Minireview Article

EFFECT OF GALANGAL RHIZOME FOR THE HEALTH OF CULTURED FISH

Abstract

For fish farmers, disease attacks 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 effort to prevent disease, to rehabilitation and also to increase endurance and health. The phytochemical content on galangal rhizome, namely phenolics/tannins, quinones, steroids/triterpenoids, flavonoids. Based on the phytochemical content, galangal rhizome has several pharmacological activities that are useful for human health, namely as antibacterial, anti-fungal, anti-inflammatory, anti-hepatotoxic, antioxidant, immunostimulant, anti-ulcerative, anti-tumor, and anti-allergic activities. In addition, galangal rhizomes can improve 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 above studies, galangal rhizome is potential and effective for maintaining fish health, because the antibacterial properties of legkuas rhizome can treat several types of cultured fish *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, Kryptoterus bicirrhis, Osphronemus gouramy* and *Borbodes gonionotus* from attack by the fungus *Saprolegnia* sp. As an immunostimulant, galangal rhizome is able to prevent eggs and fry of *C. gariepinus* from being attacked by *Saprolegnia* sp, *pseudomnas* sp. and *A. hydrophila.*

Keywords : Disease, galangal 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. Cases of disease in fish can be caused by several infectious and 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, chloramphenicol, erythromycin, kanamycin, and rimfamicin [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 immunostimulants 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 contains 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 (Figure 1) belongs to the Zingiberaceae family originating from tropical Asia spread across several countries including Malaysia, Thailand, Indonesia, Egypt, Sri Lanka, Arabic Gulf areas and China. *Alipinia galanga* is known by several regional names, including laos/laja/galangal (Indonesia), greater galangal (English), kulanjan (India), dhumarasmi (Canada), galanga (France), Kha (Thailand), hong dou ku (China) and grote galanga (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, Maranta galanga L., Amomum galanga (L.) Lour, and Amomum medium Lour [9,10]. The classification of galangal (*Alpinia galanga*) is as follows:

Kerajaan : Plantae
Divisio : Magnoliophyta
Class : Liliopsida
Ordo : Zingiberales
Family : Zingiberaceae
Subfamily : Alpinioideae
Tribe : Alpinieae
Genus : Alpinia
Species : *Alpinia galanga* (11)

Figure 1. Galangal Plants and Rhizomes
Source: darsatop.lecture.ub.ac.id

Galangal is a long-lived plant, grows in open and sunny places, 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 flesh of old rhizomes is coarse fibrous, has a sharp, pungent taste with a fragrant smell [12, 13, 14].

3. THE CONTENT AND BENEFITS OF LENGKUAS

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-labdiene-1510.25, Galanolactone, 16-dial, 12-labdiene-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 (*Alpinia galanga*) which has various biological activities [21].

Galangal contains several useful compounds, traditionally galangal rhizome has long been used as a spice for food products and also as a good source of essential oil [9, 22]. In addition, the flowers and young leaves can be used as vegetables [9]. Traditionally, galangal is often used as a medicine for stomach pain, carminative, fever, rheumatism, liver medicine, diabetes, anti-itch, anti-inflammatory, hypo-allergenic and anti-hypoglycemic, ulcers, cholera and even HIV [23, 24, 19, 9]. The pharmacological activity of the galangal plant is as antibacterial, antifungal, antiviral, antiprotozoal [25, 26, 27], immunomodulator, antioxidant effect, antidiabetic, antiplatelet, hypolipidemic, antitumor, antihelmintic, antidiuretic and pharmacological effects others [20, 24].

4. EFFECT OF GALANGAL RHIZOME ON FISH HEALTH

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 hydropila* (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 using galangal rhizomes 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 killing power against *A hydrophila* bacteria. The minimum concentration of galangal rhizome juice which has killing power against *A. hydrophila* at a density of 10^6 CFU/ml is 50% (0.835 g/mL). Other bacteria such as *Escersia coli, Staphylococcus aureus, Salmonella Bacillus subtilis* and *Staphylococcus aureus* can be inhibited by using galangal rhizomes. The inviro 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 is 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 bacteria are in the division stage, where the phospholipid layer around cells is very thin so that phenol can penetrate optimally and damage microbial cell membrane. which works as an antibacterial by denaturing proteins and damaging the microbial cell membrane. 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 (*Alpina purpurata*) showed antibacterial activity that was bacteriostatic against pathogenic bacteria in fish, 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 (*A. purpurata*) had anti-bacterial compounds including flavonoids, tannins and alkaloids. red 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 is damaged and microbial metabolism is inhibited [34]. While 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 undergoes lysis [36]. Tannins work by 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 proteins in bacterial cells experience denaturation and bacterial metabolism will be disrupted [37]. In vivo test results from several researchers showed that galangal rhizome can cope with several types of cultivated consumption fish, such as carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and 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 tilapia (O. niloticus) 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*) seeds infected with Aeromonas hydrophila bacteria with a density of 10^8 CFU/mL experienced healing after being treated with 96% ethanol extract of galangal (*A. galanga*) rhizome by soaking for 24 hours. The concentration of 385.88 ppm is an effective concentration [38].

Antifungi Activity

Galangal rhizome also has activity as an anti-fungal. 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 content of alkaloids in inhibiting the growth of the fungus Candia 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]. In vitro test results of galangal extract (*A. galanga*) using methanol and ethyl acetate solvents can inhibit the growth of *Candida albicans* fungus, whereas using hexane solvents cannot inhibit the growth of *C. albicans* fungus, this occurs because the most active fraction is in the extract of the galangal plant (*A. galanga*) is to use methanol solvent which is equal to 4.70%, while ethyl acetate is 1.70% and hexane solvent is 0% [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 lais fish (*Kryptoterus bicirrhis*) 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 gouramy*) 5 – 8 cm in size which were infected with *Saprolegnia* sp. through immersion for 12 days, with a healing percentage of 67.14 ± 0.58% [45]. Another study showed that a 100 ppm concentration of galangal rhizome (*A. galanga*) could significantly treat tawes fish (*Borbodes 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].

Immunostimulant activity

Galangal not only has antibacterial and anti-fungal 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 Yunike]. 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 invivo test results showed that galangal extract could not only increase the resistance of fish seeds to pathogenic bacteria, but also increase the resistance of fish eggs to pathogenic bacteria

and hatchability 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 challenged with *Saprolegnia* spp., *A. hydrophyla* and *Pseudomonas* sp., resulting in a higher hatchability compared to the control, which was 76.30 - 97.16%. Eggs soaked in 800 ppm extract solution and challenged with Saprolegnia 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* sp. [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 [48]. The ethanol extract of *A. galanga* showed the strongest inhibitory effect against *Streptococcus aereus* [49]. Observation of clinical symptoms after administration of the extract and before the challenge test showed that treatment using A. galangal 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 decreased and lethargic, hemorrhagic on the head, 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].

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 cultivated fish *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, Kryptoterus bicirrhis, Osphronemus gouramy* and *Borbodes gonionotus* fish from attack by the fungus *Saprolegnia* sp. As an immunostimulant, galangal rhizome is able to prevent eggs and seeds of C. gariepinus from being attacked by bacteria *Saprolegnia* sp., *pseudomnas* sp. and *A. hydrophila.*

REFERENCES

1. Sumayani, Kusdarwati R and Cahyoko Y. Antibacterial Activity of Galangal Rhizome Juice in Different Concentration to The Growth of *Aeromonas hydrophila* With In Vitro Method. Berkala Ilmiah Perikanan. 2008; 3 (1) : 83 – 87.
2. Saparinto C. Pemakaian Antibiotik Dilarang Dalam Budidaya Ikan dan Udang. 2002. www. Agritekno.tripod.
3. Haghighi M, Rohani MS, Samadi M, Tavoli M, Eslami M, Yusefi R. Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). Int J Adv Biol Biom Res. 2014; 2(6):2143-2154.
4. Kayansamruaj P, Areechon N, Unajak S. Development of fish vaccine in Southeast Asia: A challenge for the sustainability of SE Asia aquaculture. Fish and Shellfish Immunology. 2020;103:73-87.
5. Sari ETP, Gunaedi T, Indrayani E. Control of Aeromonas hydrophila Bacterial Infection in Tilapia (*Oreochromis niloticus*) with Red Galangal Rhizome Extract (*Alpinia purpurata*). Papuan Biology Journal. 2017 : 9 (2) : 37–42.
6. Mustofa V, Said NS, Fahrodi DU, Sukoco H. Zingiberaceae as A Potential Nutraceutical For Aquaculture. Samakia: Jurnal Ilmu Perikanan. 2022; 13 (2} : 119-133.
7. Matsuda, H., Pongpiriyadacha, Y., Morikawa, T., Ochi, M., & Yoshikawa, M. (2003). Gastroprotective effects of phenylpropanoids from the rhizomes of *Alpinia galangal* in rats: structural requirements and mode of action. European journal of pharmacology, 471(1), 59-67.
8. Khare CP. *Alpinia galanga* – An Important Medicinal Plant: A review. A Dictionary of Indian Medicinal Plant, Published by Springer India Pvt. Ltd. 2007;37.
9. Arambewela L, Wijesinghe A. Sri Lankan Medicinal Plant Monographs and Analysis - Alpinia galanga. 2006.
10. Sinaga E, Rahayu SE, Wahyuningsih E and Matondang I. Catalog of Plants in Indonesia. Zingiberaceae. Publisher National University Press. Jakarta. 2000. 104 pages.
11. Udjiana S. Food preservation efforts using galangal extract. J Sep Technol. 2008; 1(2).
12. Gupta RK. Medicinal and Aromatic Plants. 1st ed. CBS Publisher and Distributors Pvt. Ltd. New Delhi. 2010:468-9.
13. Kaushik D, Yadav J, Kaushik P, Sacher D, Rani R. Current pharmacological and phytochemical studies of the plant *Alpinia galanga*. Chin J Integr Med. 2011; 9(10): 1061-5. 25.
14. Scheffer J. Monoterpenes in the essential rhizome oil of *Alpinia galanga* (L.) Willd. Sci Pharm. 1981; 49: 337-46.
15. Kusriani RH, Az Zahra S. Phytochemical Screening and Determination of Total Phenolic Compounds Extracts of Red Galangal Rhizomes and White Galangal Rhizomes (*Alpinia Galanga* L.). Proceedings of the National Seminar on Research and Community Service. 2015 : 295 – 302.

16. Kubota K, Ueda Y, Yasuda M and Masuda A. Occurance and Antioxidative activity of 1'-Acetoxychavicol Acetate and its Related Compound in The Rhizomes of *Alpina galanga* During cooking. Spec Publ R Soc Chem. 2001; 274: 601 – 607.
17. Pal Jain A, Singh Pawara R, Lodhia S, Singhaia A. Immunomodulatory and antioxidant potential of Alpinia galanga Linn. rhizomes. Pharmacogn Commun. 2012;2(3):30-7. doi:10.5530/pc.2012.3.7. 7.
18. Yu JG. Identification of the chemical components of two Alpine species, Zhongyao tong bao Beijing, China. 1981;13(6):34-6.
19. Darmawan DA. The Effectiveness of White Galangal (*Alpinia Galanga* L.Willd.) Ethanol Extract in Inhibiting the Growth of Candida Albicans In Vitro. Final Assignment, Dental Education Study Program, Faculty of Medicine, University of Brawijaya. 2013.
20. Chudiwal Ak, Dp Jain. *Alpinia galanga* Wild. An overview on phyto-pharmacological properties. India. Singhad College Of Pharmacy; 2010.
21. Baradwaj RG, Rao MV, Senthil Kumar T. Novel purification of 1'S-1'-Acetoxychavicol acetate from *Alpinia galanga* and its cytotoxic plus antiproliferative activity in colorectal adenocarcinoma cell line SW480. Biomed Pharmacother. 2017;91:485-93. doi:10.1016/j.biopha.2017.04.114.
22. Jirovetz L, Buchbauer G, Shafi MP, Leela NK. Analysis of the essential oils of the leaves, stems, rhizomes and roots of the medicinal plant *Alpinia galanga* from southern India. Acta Pharm. 2003;53(2):73-82.
23. Ramesh KV, Garima S, Pradeep S, Jha KK, Khose RL. *Alpinia galanga* _ an important medicinal plant: a review, Der Pharm. Sin. 2011;2(1):142-54.
24. Verma RK, Mishra G, Singh P, Jha KK, Khosa RL. Antidiabetic activity of methanolic extract of *Alpinia galanga* Linn. aerial parts in streptozotocin induced diabetic rats. Ayu. 2015; 36(1): 91-5.
25. De-Pooter HL, Omar MN, Coolsaet BA, Schamp NM. The essential oil of greater galanga (*Alpinia galanga*) from Malaysia. Phytochemistry.1985;24(1):93-6. Doi:10.1016/S0031-9422(00)80814-6.
26. Kiuchi F, Keniji M, Itano Y, Ito M, Honda G, Qui TK. Screening of Natural Medicines Used in Vietnam for Trypanocidal Activity. Nat Med. 2002;56(2):64-8.
27. Subroto. (2006). Indonesian Medicinal Plants. 43. Volume 1. Directorate General of Drug and Food Control, Ministry of Health of the Republic of Indonesia. Jakarta.
28. Yuharmen, Eryanti, Nurbalatif. 2002. Antimicrobial Activity Test of Essential Oils and Methanol Extract of Galangal (*Alpinia galanga*), FMIPA. Riau University. Riau.
29. Florence. (2012). The Effect of Galangal Extract on Milkfish Soaking on Bacterial Amount. Jornal of Life Science, Vol.1 No.2/2012 : 114- 117.
30. Noventi W, and Novita C. Potential of green betel leaf extract (*Piper betle* L.) as an alternative therapy for Acne vulgaris. majority. 2016; 5(1): 140-145
31. Singha PK, Roy S, Dey S. Antimicrobial activity of *Andrographis paniculata*. Fitoterapia. 2003; 74: 692-694.
32. Nurhikmayani R, Daryono BS and Retnaningrum E. Isolation and molecular identification of antimicrobial-producing lactic acid bacteria from chao, South Sulawesi (Indonesia) fermented fish product. Biodiversitas. 2019; 20(4) : 1063–1068.

33. Nirwana I, Rianti D, Soekartono RH, Listyorini RD and Basuki DP Antibacterial activity of fig leaf (*Ficus carica* Linn.) extract against *Enterococcus faecalis* and its cytotoxicity effects on fibroblast cells. Veterinary World. 2018; 11(3) : 342–347.
34. Isramilda, Sahreni S and Saputra AI. Test the concentration of inhibition power of srikaya leaf decoction (*Annona squamosa* L.) on the growth of Staphylococcus aureus BEST. Journal of Biology Education, Science & Technology. 2020; 3(1) :1–8.
35. Ariani N and Riski A. Activity of the ethanol extract of kepok banana peel (*Musa paradisiaca* forma typica) on the growth of Candida albicans in vitro. Journal of Pharmascience. 2018; 5(1) :39–44.
36. Mogana R, Adhikari A, Tzar MN, Ramliza R and Wiart C. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* miq. Against bacterial clinical isolates. BMC Complementary Medicine and Therapies. 2020; 20 :55.
37. Setyowati D. Effectiveness of Galangal Extract (*Alpinia galanga*) for the Treatment of Catfish (*Clarias gariepinus*) Infected by *Aeromonas hydrophila*. Thesis. Fisheries study program. Faculty of Fisheries and Marine Science. Padjadjaran University. 2023 : 47 pages.
38. Hidayati DA, Prajitno A, Sulistyawati TD, Pratama G and Nilakandhi T. Antibacterial Activity of Red Galangal (*Alpina purpurata*) Extract on the Growth of *E. tarda* Bacteria. Journal of Aquaculture and Fish Health. 2023;12(1) : 127 -134.
39. Pratiwi, S.T., (2008). Pharmaceutical Microbiology. Jakarta: Erlangga Publisher. Pages: 188-191, 135-137.
40. Kusumaningtyas E, Widiati R. and Gholib D. (2008). Inhibition test of betel leaf extract and cream (*piper betle*) against *C. albicans* and *Trichophyton mentagrophytes*. National Seminar on Animal Husbandry and Veterinary Technology. Yogyakarta 11-10 March 2008.
41. Rahayu, P. (2013). Minimum inhibitory concentration (MIC) of star fruit (*Averrhoa bilimbi* L.) on the growth of Candida albicans. Thesis. published. Macassar. Hasanuddin University.
42. Selviana E., T. Handayani, Lilia, 2009. Determination of Galangal Concentrations to Overcome Fungal Diseases in Tilapia. Thesis. Palangkaraya. Unpar.
43. Kris B, Ardianor. Determination of the Right Concentration of Galangal Medicinal Plants in Overcoming Fungal Diseases in Domesticated Lais Fish. Thesis. Palangkaraya. Unpar. 2009.
44. Susanto E, Sidabalok I, Dewantoro E. Effect of Galangal Extract (Alpinia galangal) To Cure Carp Fish (Osphronemus gouramy) Infected By Saprolegnia sp. Ruaya Journal. 2014; 2:23 -28.
45. Saputra A. 2018. Activity Test of Galangal (*Alpinia galanga* L) as an Antifungal Saprolegnia sp. on Tawes Fish (*Borbodes gonionotus*). thesis. Pontianak Muhammadiyah University.
46. Yunike T. Galangal Builds Immunity. Trubus Magazine. 2020
47. Bendjeddou D, Lalaoui K, Satta D. Immunostimulating activity of the hot watersoluble polysaccharide extracts of *Anacyclus pyrethrum, Alpinia galanga* and *Citrullus colocynthis.* J Ethnopharmacol. 2003;88(2):155-60.

48. Saptiani G, Pebrianto CA and Hardi EH, "Anti-microbial of *Alpinia galanga* extracts against the pathogen of *Clarias batrachus*" in Inter Symp on Marine and Fisheries Research (ISMFR) Proceeding, edited by A. Isnansetyo et al. (Gadjah Mada University, Yogyakarta 2015), pp. 99-104.
49. Oonmetta-area J, Suzukib T, Gasalucka P and Eumkeb FG. Antimicrobial Properties and Action of Galangal (*Alpinia galanga* Linn.) on Staphylococcus aureus. LWT Food Sci. Tech. 2006; 39: 1214-1220.