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Antimicrobial activity of garlic and *Kaempferia galanga* on *Aspergillus* sp. growth isolated from sardine fish *Pedetan*

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Antimicrobial activity of garlic and *Kaempferia galanga* on *Aspergillus* sp. growth isolated from sardine fish *Pedetan*

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Abstract. 'Pedetan' is a traditional food made from lemuru in Bali. The existence of climate change causes changes in the quantity of fish in the waters of the Bali strait. However, the quality of the 'pedetan' must be improved.. This study aims to determine the antimicrobial activity of garlic and *Kaempferia galanga* in inhibiting the growth of *Aspergillus* sp. which can contaminate the sardine fish Pedetan. The study was conducted by experimental method by isolating *Aspergillus* sp. from Pedetan and testing the inhibitory power of garlic and *Kaempferia galanga* and the Minimum Inhibitory Concentration (MIC) of *Aspergillus* sp. The results showed that the extract of garlic and *Kaempferia galanga* can inhibit the growth of Aspergillus sp. Garlic extract can inhibit *A. flavus* with inhibition zone diameter of 20.65 mm and *Kaempferia galanga* extract can inhibit the inhibition zone diameter of 23.75 mm. Minimum Inhibitory Concentration (MIC) for garlic extract was 0.4% with inhibition zone diameter of 1.0 mm and *Kaempferia galanga* with inhibition zone diameter of 1.2 mm. Garlic extract and *Kaempferia galanga* at 0.5% concentration can inhibit 100% of the conidia *Aspergillus* sp. Garlic extract and *Kaempferia galanga* can inhibit the growth of *Aspergillus* sp. because they contains bioactive compounds that are antimicrobial.

1. Introduction

The existence of climate change causes changes in the quantity of fish in the waters of the Bali Strait. The phenomenon of sardine fish which was lost in 2014-2017 has had an impact on the coastal communities of Bali. Hundreds of sardine fishing fishermen have drastically decreased their income. The sardine industry which relies on sardine fish is also having difficulty finding raw materials. Now sardine fish are again in abundance. This is marked by the re-capture of sardine by Pengambengan fishermen, around March 2018. Sardine fish are abundant around the Bali Strait from September to November. In 2018 the sardine landed in Pengambengan reached 1,154.1 tons, with a production value of Rp. 5.85 billion. Until 2019 the production volume of sardine fish has reached 4,217.8 tons. There was an increase of 365 percent compared to 2018. The production value of sardine fish until the end of July 2019 reached IDR 20.2 billion or 248 percent greater than the production value throughout 2018 [1].

The traditional food product for *pedetan* is processed from sardine fish in Jembrana Regency, Bali Province. The community processes and extends the sardine fish's shelf life by processing it into food products that can be stored longer, commonly referred to as *pedetan* (Figure 1). *Pedetan* is made from sardine fish, salt and spices (coriander, galangal, garlic and kaemferia), dried in the sun for two to three days, then stored at room temperature [2].

Dry fish damage can occur during storage and during distribution in marketing. Some damage that may occur in dried fish, among others: Damage due to microorganisms, such as bacteria and fungi,

Damage caused by enzymes, both enzymes derived from fish and from bacteria. The spices used in the process of making sardine fish 'spicy' can be used as one of the inhibiting ingredients for damage to dried fish processed products. Garlic contains the active compound allicin which has anti-microbial activity [3].

Figure 1. *Pedetan* of sardine fish.

Processing of sardine fish *pedetan* is done by the manufacturer uses a mixture of several different spices depending on the taste of the local community. The ingredients that are always used in making *pedetan* are kaemferia and garlic. Garlic can inhibit the growth of *Salmonella enteritidis* and *Staphylococcus aureus* [4]. Allicin and organosulphur compounds in garlic can inhibit the growth of gram-positive bacteria and gram-negative bacteria such as *Staphylococcus, Salmonella, Vibrio, Mycobakteria, Proteus* sp, *Helicobacter pylori* and also as anti-parasite, antifungal and anti-virus [5][6]. Kaemferia extract (*Kaempferia galanga* L.) can inhibit the growth of *Escherichia coli* [7]. The inhibitory effect of kaemferia extract on *Trichophyton tagrophytes* and *Cryptococcus neoformans* by diffusion method showed that the kaemferia extract was able to inhibit the growth of *Tri-chophyton mentagrophytes* and *Cryptococ-cus neoformans* with a minimum inhibitory extract concentration of 0.15% against *Tri-chophyton mentagrophytes* and 2% against Cryptococcus neoformans [8]. Therefore it is necessary to do research to analyze the ability of garlic and kaemferia in inhibiting mold *Aspergillus* sp. in the sardine fish.

2. Materials and methods

2.1. Sampling and extraction methods

Pedetan samples were taken at the state market of Jembrana Regency, Bali, then the *Pedetan* samples were weighed 1 g and carried out a series of dilutions from 10^{-1} to 10^{-6}. Furthermore, at 10^{-6} dilutions in each sample, 100 µL of the suspension was taken and put into 10 mL of PDA media in a Petri dish and incubated at room temperature for 2 days. *Aspergillus* sp. isolates were obtained. Based on the morphology of mold growth and based on the alignment of the 18S rRNA gene sequence with the GenBank database using the BlastN program [9].

Garlic and kaemferia are dried for 2-3 days until constant weight is obtained. Dry samples are blended until they become powder. Dry samples of 1000 g were macerated with 2000 mL of 70% ethanol for 24 hours 3 times. The filtrate obtained was combined and evaporated with a rotary vacuum evaporator, so that crude extracts of ethanol were obtained. The extract is used for further testing.

2.2. *Determination of minimum inhibitory concentration (MIC)*

Suspension of mold spores *Aspergillus* sp. (200 µL) is put into a Petri dish then mixed with 10 mL PDA media. Petri dishes are rocked horizontally so that the mold suspension is evenly mixed. After solid, a diffusion well with a diameter of 5 mm was made of 2 pieces per Petri dish using cork borer. Each diffusion well was filled with 20 μL of crude extract of garlic and kaemferia with a concentration of 10%. This culture was incubated in a dark place at room temperature and the formation of a barrier zone around the diffusion well was observed every day for 5 days. The test to find out the Minimum Inhibitory Concentration (MIC) was also carried out by the diffusion well method with several extract concentrations, namely: 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6 %, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5% and 0% control [10].

2.3. *Inhibitory power test for biomass growth*

Spore formation testing using Completely Randomized Design with 2 factors, the first factor was the mold type which consisted of 4 types of mold and the second factor was the concentration of extract consisting of 5 treatment concentrations of crude extract 0%, 0.1%, 0.2%, 0.3%, 0.4 and 0.5%, the experiment was carried out with 4 repetitions. The test is done by growing spores on the PDB media in 200 mL Erlenmeyer volume. Seasoning extract was put into Erlenmeyer according to the concentration tested, then it was put into 1 mL mold spore suspension (10^6 spores/mL). The final culture volume (PDB + extract + mold spore suspension) is 100 mL, then shaken so that the extract is evenly mixed with the media.

Incubation was carried out at 30°C and shaker at a speed of 100 rpm for 8 days. On the eighth day the mold biomass is separated by PDB media by centrifuging at 5,000 rpm for 3 minutes. The deposits obtained were dried at a temperature of 60°C until the weight was constant. Biomass is weighed and weighed compared to the weight of control mold biomass.

3. Results

3.1. *Results of testing the single seasoning inhibitory Aspergillus sp.*

The results of the anti-mold activity test of spices extract against mold *Aspergillus* sp. showed that garlic and kaemferia were able to inhibit the growth of mold *Aspergillus* sp. with inhibition zone diameter of 20.65 mm and 23.75 mm (Table 1 and Figure 2).

Table 1. Test of antique activity of seasoning extract on mold *Aspergillus* sp.

Spice	Inhibit (mm)
Garlic	20.65
Kaemferia	23.75

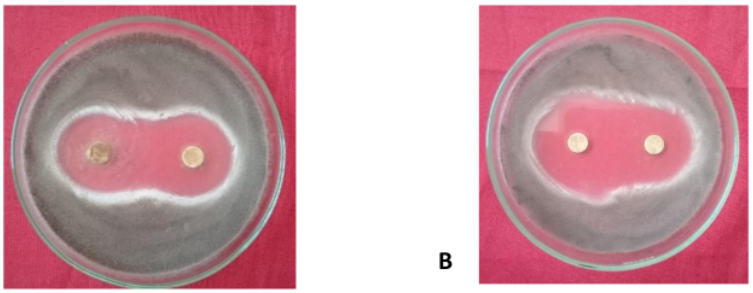

Figure 2. A. Inhibit Garlic extract to *Aspergillus* sp. B. Inhibit Kaemferia extract to *Aspergillus* sp.

3.2. Observation data minimum inhibitory concentration (MIC)

The results showed that the extract of garlic and kaemferia significantly ($p < 0.05$) showed that the minimum concentration that could cause barriers (MIC) to the growth of *Aspergillus* sp. on PDA media was 0.4% with inhibition zone diameter by garlic by 1.0 mm, and by kaemferia by 1.2 mm (Table 2). The minimum inhibitory power shows a significant difference in 0.4% extract concentration against garlic and kaemferia.

Table 2. The results of testing the MIC of garlic extract and *Kaemferia galanga* against *Aspergillus* sp.

Extract concentration	Inhibit (mm)	
	Garlic	Kaemferia
0.1	0.0 a	0.0 a
0.2	0.0 a	0.0 a
0.3	0.0 a	0.0 a
0.4	1.0 b	1.2 b
0.5	1.0 b	1.2 b
1.0	1.0 b	1.2 b
1.5	1.2 c	1.3 c
2.0	1.2 c	1.3 c
2.5	1.2 c	1.3 c
3.0	1.4 d	1.4 d
3.5	1.5 e	1.4 d
4.0	1.5 e	1.5 e
4.5	1.5 e	1.5 e
5.0	1.6 f	1.5 e

* The average value followed by the same letter in the same column shows a non-significant difference ($p > 0.05$) according to Duncan's Multiple Range Test

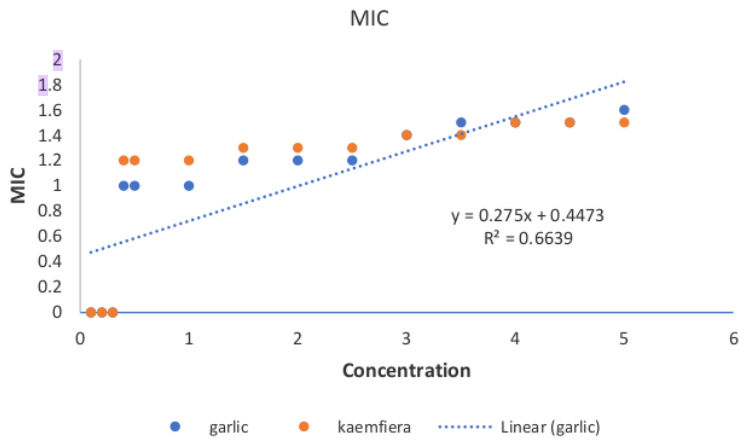

Figure 3. Graph of the relationship of spice extract with minimum growth inhibition (MIC) of *Aspergillus* sp.

The relationship between spice extract with minimum growth inhibitory power of *Aspergillus* sp. shows the linear equation y = 0.275 + 10.4473, with the determination value R^2 = 0.6639 which means that the spice extract affects the minimum growth inhibition of mold *Aspergillus* sp (Figure 3).

3.3. Biomass growth observation data

The results showed that the extract of garlic and kaemferia significantly (p<0.05) inhibited the dry weight of the mold biomass *Aspergillus* sp. The treatment of crude extract with 0.1% concentration of garlic can inhibit the dry weight of *Aspergillus* sp. ranged from 18.32% to 94.80% when compared to controls. The treatment of coarse extract with 0.1% concentration of kaemferia was able to inhibit the dry weight of fungal mycelia *Aspergillus* sp. ranged from 13.84% to 96.61% when compared to controls (Table 3).

Table 3. The inhibitory power of Garlic extract and kaemferia on *Aspergillus* sp biomass.

Extract concentration	Fungi Biomassa		Inhibit (%)	
	Garlic	Kaemferia	Garlic	Kaemferia
0	1.097 a	1.120 a	-	-
0.1	0.896 b	0.965 b	18.32	13.84
0.2	0.109 c	0.187 c	90.06	83.30
0.3	0.096 d	0.075 d	91.25	93.30
0.4	0.057 e	0.038 e	94.80	96.61
0.5	0.000 f	0.000 f	100.00	100.00

* The average value followed by the same letter in the same column shows a non-significant difference (p> 0.05) according to Duncan's Multiple Range Test.

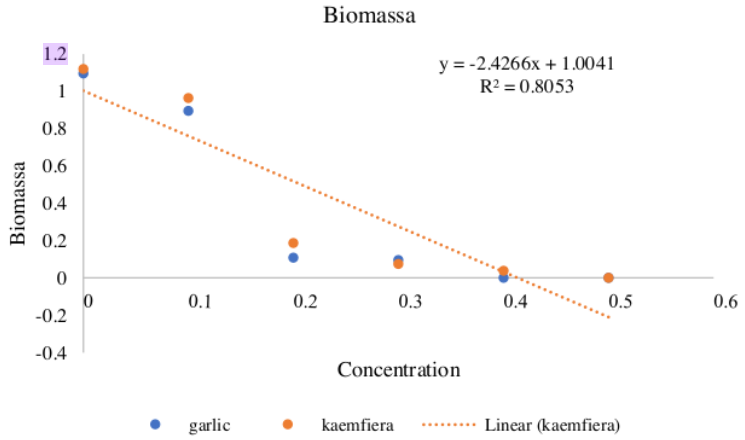

Figure 4. Graph of the relationship of seasoning extract with biomassa *Aspergillus* sp.

The relationship between spice extract with *Aspergillus* sp. biomass growth inhibitory power shows the linear equation y = 2.4266x + 1.0041, with the determination value R2 = 0.8053 which means that the seasoning extract has an effect on the inhibitory power of *Aspergillus* sp (Figure 4).

4. Discussion

Several studies show that the spices used for *Pedetan* have antiperspirant and antibacterial activity [9]. Garlic has an antimicrobial effect on Gram positive and Gram negative bacteria, molds and parasites

[5]. The garlic can inhibit the growth of *Salmonella enteritidis* and *Staphylococcus aureus* [4] and was able to inhibit the growth of *Candida albicans* [11].

Garlic contains the active compound Allicin which has anti-microbial activity. Allicin in the form of pure compounds has the ability of Gram positive and Gram negative antibacterial activity, bacterial species that can be inhibited by garlic extract include *Staphylococcus aureus, Hemolytic streptococcus* α and β, *Citrobacter freundii, Enteroccocus cloacae, Enterpbacter cloacae, Escherichia coli, Proteus vulgaris , Salmonella enteritidis, Citrobacter, Klebsiella pneumonia, Mycobacteria, Pseudomonas aeruginosa, Helibacter pylori* and *Lactobacillus odontyliticus*, several types of fungi such as *Candida albicans*, *Aspergillus niger, Saccharomyces cerevisiae, Fusarium oxysporium* [12].

The *Kaemferia rhizome* can inhibit pathogenic bacteria such as *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Pesudomonas aeruginosa* and *Vibrio cholerae* and four mold species, namely *Aspergillus niger, A. flavus, A. fumigatus* and *Candida albicans*. All extracts show significant antibacterial and anti-mild properties. The highest inhibition zone (21.3 ± 0.08) was recorded for ethanol extract against *Staphylococcus aureus* [13].

Kaemferia extract showed anti-mold activity against *Aspergillus niger, Candida albicans* with inhibitory zones of 11-22 mm, higher than that of gram-negative bacteria *Enterobacter aerogenes, Escherichia coli*, namely 9.5-15 mm and gram-positive bacteria *Bacillus subtilis, Klebsiella pneumonia, Serratia marcescens*, and *Pseudomonas aeruginosa* is 9.5-13 mm. So that the *Kaemferia rhizome* extract showed good antioxidant and antimicrobial activity.

The results showed that the seasoning extract contained alkaloids, terpenoids, flavonoids, and phenolics where these compounds contributed to inhibiting the growth of mold Aspergillus sp. so that it will affect the dry weight of the mold mycelia. The results of this study are in line with the results of the research [14] that the treatment of coriander oil, fennel and caraway with a concentration of 500 ppm is able to inhibit the dry weight of *A. flavus* strain ATCC 16872. *C. sinensis* oil at concentrations of 1.5 µg/mL, 2 µg/mL, and 2.5 µg/mL were able to inhibit the dry weight of A. niger fungi by 91.70%, 95.77%, and 100% respectively compared to controls [15].

Fenugreek ethanol extract at a concentration of 1% was able to inhibit the dry weight of *A. flavus* and *A. parasiticus* molds by 14.1% and 14.1% when compared to controls [16]. While the *Cymbopogon martini* extract at a concentration of 0.75 µl/mL was able to inhibit the dry weight of *A. flavus* fungi mycelia strains ATCC 13697 and *A. niger* strain of ATCC 10535 with a percentage of inhibition of 71.18% and 91.85% if compared to controls [17]. The higher the concentration of the spice extract used, the higher the inhibitory power of fungi biomass *Aspergillus* sp. From the results of observations of SEM (Scanning Electron Microscope) with a magnification of 3,000 and a bar of 5 µm. it was found that garlic and galangal were able to damage the mycelia of Aspergillus sp. compared to control treatment [18].

5. Conclusion

Garlic extract and kaemferia can inhibit the growth of mold colonies *Aspergillus* sp. which pollutes the sardine *pedetan*. At 0.4% extract can minimum inhibitory concentration (MIC) to the growth of *Aspergillus* sp. The treatment of crude extract with 0.1% extract concentration was able to inhibit the dry weight of *Aspergillus* sp.

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