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The Ability of Seasoning to Inhibit Contaminant *Aspergillus* spp. on Traditional Food Sardine *Pedetan* (*Sardinella lemuru*)

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Abstract

Pedetan is processed food of seasoned sardine fish (*Sardinella lemuru*) which is preserved by drying. Isolation and identification of mold that contaminated the *pedetan* have been taken from 10 villages which are production centers in Jembrana Regency. Molds that can contaminate *pedetan* during storage, include *Aspergillus flavus, Aspergillus aculeatus, Aspergillus niger*, and *Aspergillus tubingensis*. In this research, the composition of the seasoning from the producer was used to inhibit the growth of contaminant fungi. The minimum antimicrobial inhibition test was carried out by the well diffusion method and the seasoning extract was tested for its inhibition on mold growth on potato dextrose agar (PDA) media. Mold colonies growth test and testing the effect of extracts on mold biomass were carried out *in vitro* with potato dextrose broth (PDB) media. The results showed that the composition of seasoning garlic, coriander, kaempferi, galangal, ginger, vinegar, and salt can inhibit *Aspergillus* spp. Seasoning extract concentrations of 0.5 % (w/v) diameter of inhibitory zone minimum inhibitory concentration (MIC) are 0.8 and 1.2 mm for *A. aculeatus* and *A. niger*, by 1 mm for *A. flavus* and *A. tubingensis*, respectively. Seasoning extract at a concentration of 0.5 % (w/v) is also able to inhibit up to 100 % to the growth of the colony, conidia germination, conidia density, and biomass of *A. flavus, A. aculeatus, A. niger*, and *A. tubingensis* that contaminate the sardine fishes *pedetan*.

Keywords: Minimum inhibitory concentration, Conidia density, Conidia germination, Scanning electron microscope

Introduction

Sardine fishery resources are the most dominant and economically valuable fishery resources in Bali Strait. These commodities are most exploited by fishermen who live around Bali Strait. Sardine fishing business is a source of regional income, supporting the local industry and increasing employment, both at sea and on land. Sardine fish is known as one of the types of small pelagic fish that contains unsaturated fatty acids omega 3 fatty acids (14.38 %) and omega 6 (5.73 %) [1]. The local community of Jembrana Regency, Bali Province, uses sardine in the manufacture of fishery products, especially in the fish canning industry, boiled fish, salted fish, fish meal, and *pedetan*.

Pedetan is a food product of seasoned dried sardine fish which is seasoned [2]. Processing different from the process of receiving raw materials to the distribution process [3,4] and storage of sardine with different packaging materials will affect the quality and safety of the produced *pedetan* [5]. Mold that contaminate was taken from 10 villages which are production centers in Jembrana Regency, they were then isolated and identified. Identification was carried out macroscopically, microscopically, and molecularly through 18S rRNA gene analysis, showing that there were 4 types of molds found as pollutant contaminants, namely *Aspergillus flavus, Aspergillus aculeatus, Aspergillus niger*, and *Aspergillus tubingensis* [6]. Dried smoked fish microflora showed a molds growth of $1.2\times10^{3} - 2.6\times10^{3}$ CFU/g [7]. Molds were characterized and identified as *A. niger, A. flavus, A. fumigatus, Fusarium solani, Mucor mucedo*, and *Saccharomyces cerevisiae*. Dried smoked fish can also be contaminated by

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Fusarium, Aspergillus, Saccharomyces, Penicillium, Mucor, Rhodotorula, Schizosaccharomyces, Acremonium, and *Rhizopus*. Out of 16 mold isolates, only *Aspergillus flavus* (8.33 %) has the potential to produce aflatoxin. Considering the safety and quality of seafood, the presence of toxigenic molds in dried fish is important for health because it can increase the risk of mycotoxin [8]. *Aspergillus* sp. was also found in polluted corn kernels and 6 species were identified, namely *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigatus, Aspergillus clavatus,* and *Aspergillus terreus*. *Aspergillus* sp. in corn kernels can indicate the mycotoxin produced. The occurrence of toxigenic species such as *A. niger, A. flavus,* and *A. fumigatus* indicate a possible risk of mycotoxin contamination from corn kernels [9]. An attempt was made to improve the composition of the spices to be able to suppress the contaminant molds population to the *pedetan* more optimally [6].

The benefits of spice in inhibiting microbial growth have been reported in several research results. Garlic has been shown to inhibit lipid oxidation and reduce the number of microbes. Smoke sardines with concentrations of garlic extract 5, 10, and 15 g can reduce the number of total aerobic plates (CFU/g) for 30 days of frozen storage (−18 °C) [10]. Coriander oil with a concentration of 0.1 - 100 ppm can act as antimicrobial pathogenic and saprophytic microorganisms. Essential oils in coriander seeds contain 67.7 % linalool; 10.5 % a-pinene; 9.0 % g-terpinene; 4.0 % geranyl acetate; 3.0 % camphor; and 1.9 % geraniol [11]. Galangal with concentrations starting at 0.5 % can inhibit the growth of *A. flavus* by 9 mm, and at concentration of 4 %, it can inhibit up to 30 mm [12]. Galangal can inhibit the growth of molds of *Aspergillus* spp. and *F. moniliforme* [13]. *Zingiber officinale* Roscoe (ginger) has the potential to treat and prevent diabetes. It has been tested in humans and animal experiments. An increase in glucose transport by ginger extract at 400 µg/mL is accompanied by an increase in protein expression [14]. Kaemfiera can inhibit pathogenic bacteria such as *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Vibrio cholerae* and 4 species of molds namely *Aspergillus niger, A. flavus, A. fumigatus* and *Candida albicans* [15].

Based on the foregoing, a traditional seasoning of antifungal activity was carried out consisting of garlic, coriander, kaemfiera, salt, vinegar, galangal, and ginger and its ability to inhibit the growth of *Aspergillus* sp. which contaminants the traditional food of sardine fish *pedetan*.

Materials and methods

Sample preparation

Seasoning extracts consisting of garlic, coriander, kaemfiera, salt, vinegar, galangal, and ginger were prepared based on the composition of spices used by traditional food producers. The spices were dried for 2 - 3 days until a constant weight was obtained. The dried spice sample was crushed to powder. Dry sample powder of 1,000 g was macerated with 2,000 mL 70 % ethanol for 24 h 3 times. The obtained filtrate was combined and evaporated by the solvent with a rotary vacuum evaporator, to obtain 70 % ethanol crude extracts. The extract was used for further tests. Pollutant mold contaminants namely *Aspergillus flavus, Aspergillus aculeatus, Aspergillus niger,* and *Aspergillus tubingensis* were obtained from the Biopesticide Laboratory of Udayana University from previous research. Isolation and identification of mold that contaminated the *pedetan* have been taken from 10 villages which are production centers in Jembrana Regency. Identification was carried out macroscopically, microscopically, and molecularly through 18S rRNA gene analysis, showing 4 types of molds found as pollutant contaminants, namely *Aspergillus flavus, Aspergillus aculeatus, Aspergillus niger,* and *Aspergillus tubingensis* [6].

Antimicrobial minimum inhibitory test with well diffusion method

Testing to find out the minimum inhibitory concentration (MIC) was also carried out by the well diffusion method with different variations in the concentration of seasoning extract 0.1 - 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 % (w/v) (0 % as a control with sterile water). This dilution was done by adding sterile distillate water containing 10 % tween 80 according to concentration. Each treatment was repeated 4 times. Observations were made by measuring the diameter of the inhibition zone formed around the well diffusion [16,17].

Inhibitory test of seasoning extracts on growth of mold colonies

The seasoning extract was tested for its inhibition on mold growth on PDA media with a crude extract concentration of 0 - 0.5 % (w/v). Each concentration was made 1 mL then each of the 1 was poured into a sterile petri dish, added with 9 mL of diluted PDA media (temperature ± 45 °C), shaken

horizontally so that the extract and PDA were evenly mixed. After the media became solid, a 5-day old mold colony was taken using a 5 mm diameter cork-borer placed in the center of a Petri dish using an ose needle. Each extract concentration was made 5 replications. Subsequently, it was incubated at room temperature (27 - 30 °C), until molds in the control filled the Petri dish.

Observations were made every day by measuring the diameter of the mold at each treatment. The percentage of inhibition was calculated by comparing the growth of mold on the media given the extract and the growth of mold on the media as a control. Inhibitory treatment of extracts against colony growth was calculated using the following formula:

$$\text{Inhibitory (\%)} = \frac{\text{Diameter of control colony} - \text{Diameter of the treatment colony}}{\text{diameter of control colony}} \times 100\ \%$$

Inhibitory testing of spore formation

Testing was carried out *in vitro* by inoculating 200 µl of spore suspension (10^6 spores/mL) on 10 mL of potato dextrose broth (PDB) media. Then, each was added to spice extract with a crude extract concentration of 0 - 0.5 %. For control (0 %), it was done by adding mold suspension in PDB media without extract, but 200 µl of sterile water was added. This culture was incubated in a dark place at room temperature for 3 days. Calculation of the number of spores formed was done by hemocytometer observed through a microscope. Calculation of the inhibition against the formation of spores was calculated by using the following formula:

$$\text{Inhibitory (\%)} = \frac{\text{Spore control density} - \text{Treatment spore density}}{\text{spore control density}} \times 100\ \%$$

Testing the effect of seasoning extract on mold biomass

Testing the effect of seasoning extracts on mold biomass was carried out by growing spores on PDB media in a volume of 200 mL Erlenmeyer. Seasoning extract with a concentration of 0 - 0.5 % was put into Erlenmeyer. Then, 1 mL of mold spore suspension (10^6 spores/mL) was put into it. The final volume of culture (PDB + extract + suspension of mold spores) was 100 mL. Then, it was shaken so that the extract was mixed evenly with the media.

Incubation was carried out at a temperature of 30 °C and it was shaken at a speed of 100 rpm for 8 days. On the 8 day, the biomass of the molds was separated by PDB media by centrifugation at a speed of 5,000 rpm for 3 min. The precipitate obtained was dried at 60 °C until the weight was constant. Biomass was weighed and compared to the weight of control mold biomass. The inhibitory power of extracts from biomass formation was calculated using the following formula:

$$\text{Inhibitory (\%)} = \frac{\text{Biomass weight control} - \text{Biomass weight of treatment}}{\text{Biomass weight control}} \times 100\ \%$$

Observation of the structure of hyphae of *Aspergillus* spp. with scanning electron microscope (SEM)

Testing the effect of seasoning extract on the structure of *Aspergillus* spp. was done by adding each of the 4 *Aspergillus* spp. into the 100 mL PDB medium. Erlenmeyer shaken for 48 h at a speed of 130 rpm. After 48 h of crude extracts of 0.5 and 1 % were added to the Erlenmeyer, and continued with incubation at a temperature of 30 °C shaker for 72 h at a speed of 100 rpm. Media that is not added to the crude extract as a control was made, and each replication was made 3 times. After 72 h, the mold biomass was separated by PDB media by filtering. The precipitate obtained was dried at 50 °C until the weight was constant. The next step was the sample preparation process for scanning electron microscope (SEM) analysis.

Statistical analysis

The data of this research were analyzed quantitatively by analysis of variance (ANOVA). If the data show significant differences, Duncan's Multiple Range Test (DMRT) was performed. Statistical analysis was conducted using SPSS for Windows version. 22 of 2015.

Results and discussion

Minimum inhibitory concentration (MIC) test results of seasoning extracts

The results showed that the minimum inhibitory concentration that can cause obstacles to the growth of 4 types of molds of *Aspergillus* spp. on PDA media is 0.5 % with inhibition zone diameters ranging from 0.8 - 1.2 mm. Inhibitory zone diameter in *A. aculeatus* was 0.8 mm, in *A. niger* was 1.2 mm while in *A. flavus* and *A. tubingensis* was 1 mm (**Table 1**).

Table 1 MIC test results of seasoning extracts against *Aspergillus* spp.

Extract	Inhibitory zone diameter (mm)			
Concentration (%)	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*
0.1	0.0 ^{b*}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}
0.2	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}
0.3	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}
0.4	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}
0.5	1.0 ^{a**}	0.8 ^{b**}	1.2 ^{a**}	1.0 ^{a**}
1.0	1.0 ^{a}	1.2 ^{a}	1.3 ^{a}	1.0 ^{a}
1.5	1.4 ^{a}	1.3 ^{a}	1.3 ^{a}	1.1 ^{a}
2.0	1.4 ^{a}	1.3 ^{a}	1.4 ^{a}	1.2 ^{a}
2.5	1.4 ^{a}	1.3 ^{a}	1.4 ^{a}	1.3 ^{a}
3.0	1.4 ^{a}	1.4 ^{a}	1.4 ^{a}	1.4 ^{a}
3.5	1.5 ^{a}	1.4 ^{a}	1.5 ^{a}	1.4 ^{a}
4.0	1.5 ^{a}	1.5 ^{a}	1.5 ^{a}	1.5 ^{a}
4.5	1.5 ^{a}	1.5 ^{a}	1.5 ^{a}	1.5 ^{a}
5.0	1.7 ^{a}	1.8 ^{a}	1.7 ^{a}	1.[illegible] ^{a}

*The average value followed by the same letter in the same column shows an insignificant difference based on Duncan's Multiple Range Test at 5 % level
**MIC

Galangal, ginger, turmeric, and garlic extracts with concentrations ranging from 0.5 % can inhibit the growth of *A. flavus* by 9, 4, 4, 5 mm, respectively and at concentrations of 4 % can inhibit each of 30, 7, 9 and 6 mm [12]. MIC of garlic water extracts on *A. niger, A. fumigatus, A. terreus* were 0.4, 3.6, and 3.2 mg/mL, respectively [18]. The MIC of *Cymbopogon martini* extract in *A. niger* strain ATCC 10535 and *A. flavus* strain ATCC 13697 were 2 and 4 mg/mL [19].

Plant species and mold species affect the size of the MIC on the mold. The MIC of *Allium obliquum, Allium fistulosum*, and *Allium ursinum* plant extracts on *A. niger* molds were 80, 100, and 120 µl/mL, respectively, whereas MIC of *Allium obliquum* plant extracts were 80, 100, and 120 µl/mL. MIC of *Allium obliquum* in *Botrytis cinerea, Penicillium expansum*, and *Sclerotinia sclerotiorum* molds were 60, 80, and 50 µl/mL, respectively [20].

Inhibition of seasoning extracts against the growth of *Aspergillus* spp.

The results showed that seasoning extracts composition significantly ($p < 0.05$) inhibited the growth of the colony of 4 species of *Aspergillus* spp. on PDA media with a percentage of inhibition ranging from 30 - 100 %. Treatment of traditional herbs composition extract with a concentration of 0.1 % was able to inhibit the growth of colonies of 4 species of *Aspergillus* spp. ranging from 14 - 45.4 %. Treatment of seasoning extracts composition extract with a concentration of 0.1 % was able to inhibit the growth of *A. flavus, A. niger, A. tubingensis,* and *A. aculeatus* colonies by 30;32.5;37.6;45.4 %, respectively (**Table 2**).

Table 2 Inhibition of seasoning extract on the colony diameter of *Aspergillus* spp.

Extract concentration	Colony diameter (mm)				Percentage of inhibitory (%)			
(%)	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*
0	20.0 [a*]	22.0 [a]	20.0 [a]	34.5 [a]	-	-	-	-
0.1	14.0 [b]	12.0 [b]	13.5 [b]	21.5 [b]	30.0	45.4	32.5	37.6
0.2	10.5 [c]	8.5 [c]	9.0 [c]	14.5 [c]	47.5	61.3	55.0	57.9
0.3	6.0 [d]	5.5 [cd]	6.5 [c]	7.0 [d]	70.0	75.0	67.5	79.7
0.4	3.0 [de]	3.4 [de]	2.9 [d]	3.5 [e]	85.0	84.5	85.5	89.8
0.5	0.0 [e]	0.0 [e]	0.0 [d]	0.0 [f]	100	100	100	100

*The average value followed by the same letter in the same column shows an insignificant difference based on Duncan's Multiple Range Test at 5 % level.

The results showed that the extracts of seasoning composition contained alkaloids, terpenoids, flavonoids, and phenolic compounds in which these compounds contributed to inhibiting the growth of molds of *Aspergillus* spp. Treatment of traditional spices composition extract with a concentration of 0.5 % was able to inhibit the growth of *A. flavus*, *A. aculeatus*, *A. niger*, and *A. tubingensis* colonies by 100 % when compared to controls (**Figure 1**).

The results of this study are in line with the results of a study by [21] suggesting that ginger extract was able to inhibit the growth of *Aspergillus foetidus*, *Aspergillus versicolor*, *Aspergillus carbonarius*, and *Aspergillus nidulan* at 31, 59, 64, and 73 % when compared to controls. Ginger extract with a concentration of 15 % can inhibit the growth of mycelia *Alternaria* mold by 57 % when compared to controls [22]. The *Carica papaya* root extract with a concentration of 10 % was able to inhibit the growth of *A. flavus* and *A. niger* at 8.33 and 35 % when compared to controls [23]. The *Ocimum gratissimum* extract was able to inhibit the growth of *A. flavus*, *A. niger*, and *A. tamari* at 50, 56 and 56.3 %, respectively when compared to controls [24].

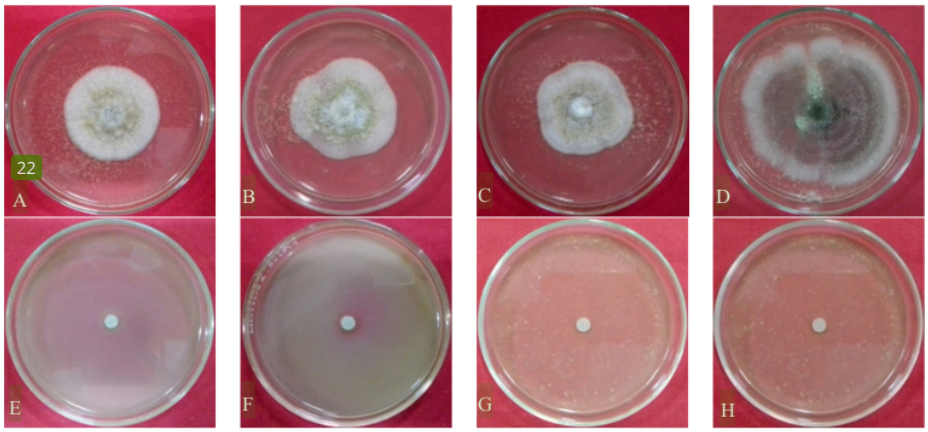

Figure 1 Test of anti-molds activity of seasoning extracts on the growth of *Aspergillus* spp. (A). Control treatment *A. flavus*, (B). Control treatment *A. aculeatus*, (C). Control treatment *A. niger*, (D). Control treatment *A. tubingensis*, while (E) - (H) are seasoning extract treatment with a concentration of 0.5 % (w/v).

Galangal, ginger, turmeric, and garlic extracts with a concentration of 0.5 % inhibitory growth of *A. flavus* colonies were 28.0;1.2;0.8;1.2 %, while at a concentration of 4 % inhibit at 44, 4.0, 2.4 and 3.2 % [12]. Coriander, galangal, garlic, and ginger extracted using 30 % hot water were able to inhibit the growth of mycelia mold *Rhizoctonia solani* with a percentage of inhibitory successively by 15.29, 21.17, 25.88 and 40.0 % when compared to controls [25]. The higher the concentration of seasoning extract used, the higher the inhibitory effect on the growth of *Aspergillus* spp. The relationship between extract concentration and inhibitory effect on colony growth followed the equation y = 156.7x + 25.99 with the coefficient of determination (R^2) = 0.9678 (**Figure 2**).

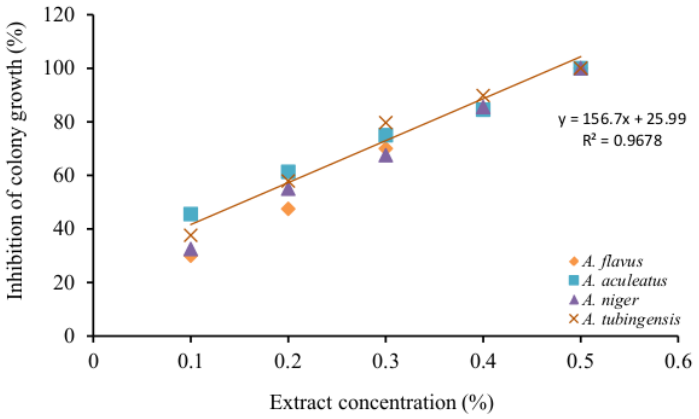

Figure 2 Graph of the relationship between the concentration of seasoning extracts with the inhibition of the growth of 4 colonies of *Aspergillus* spp.

Inhibition of seasoning extracts against conidia germination of *Aspergillus* spp.

The results showed that seasoning extract significantly ($p < 0.05$) inhibited conidia germination of 4 species of *Aspergillus* spp. The crude extract treatment with a concentration of 0.1 % was able to inhibit conidia germination of 4 types of molds of *Aspergillus* spp. which ranged from 66.9 - 75.8 % when compared to controls. The crude extract treatment with a concentration of 0.1 % was able to inhibit the conidia of *A. tubingensis, A. flavus, A. niger,* and *A. aculeatus*, respectively by 66.9, 75.7 and 75.8 % compared to controls. Whereas the treatment of crude extract with a concentration of 0.5 % was able to inhibit the conidia of *A. flavus, A. aculeatus, A. niger,* and *A. tubingensis* by 100 % compared to controls (**Table 3**).

Conidia is airborne spores that play an important role in the distribution and survival of molds. The conidia will germinate if it is on the appropriate substrate. Alkaloid compounds, terpenoids, flavonoids, and phenolics contained in the spice extract are thought to inhibit the synthesis of proteins and enzymes needed in cell formation so that they will inhibit the germination process of *Aspergillus* spp. The results of this study are in line with [26] which reported that kaemfiera can inhibit *Aspergillus niger* and *Candida albicans*. [15] reported that kaemfiera could inhibit 4 species of mold, namely *Aspergillus niger, A. flavus, A. fumigatus,* and *Candida albicans*.

Table 3 Inhibition of seasoning extracts against conidial germination of *Aspergillus* spp.

Extract concentration	Conidia germination ($\times 10^5$ conidia/mL)				Percentage of inhibitory (%)			
(%)	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*
0	92.5 [a*]	83.6 [a]	80.7 [a]	95.4 [a]	-	-	-	-
0.1	24.1 [b]	20.2 [b]	19.6 [b]	31.5 [b]	73.9	75.8	75.7	66.9
0.2	12.3 [c]	11.4 [c]	10.3 [c]	13.6 [c]	86.7	86.4	87.2	85.8
0.3	6.3 [d]	5.7 [d]	5.0 [d]	6.2 [d]	93.2	94.0	93.8	93.5
0.4	2.4 [e]	2.1 [de]	1.8 [de]	2.0 [e]	97.4	97.5	97.7	97.9
0.5	0.[illegible]	0.0 [e]	0.0 [e]	0.0 [e]	100	100	100	100

*The average value followed by the same letter in the same column shows an insignificant difference based on Duncan's Multiple Range Test at 5 % level.

The ethanol extract of *Costus speciosus* (included in the order of *Zingiberales*) with a concentration of 5 % was able to inhibit conidia growth of *A. fumigatus, A. niger, A. terreus,* and *A. flavus* at 53.81, 59.91, 62.21 and 65.77 % when compared to controls [27]. The *Citrus sinensis* oil at a concentration of 0.7, 1 and 1.5 µg/mL was able to inhibit conidia of *A. niger* at 89.8, 95.5 and 100 % [28].

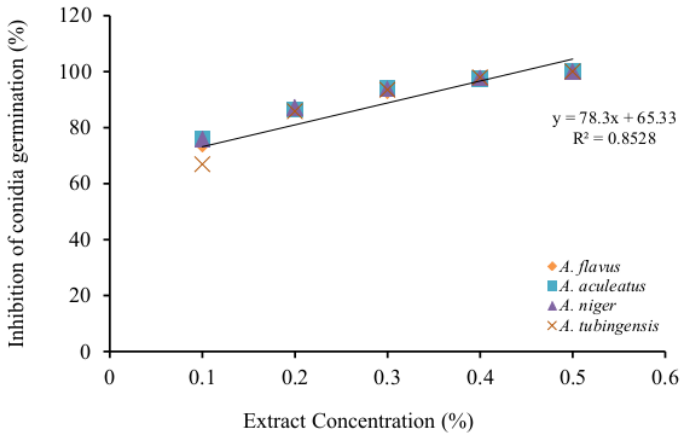

Figure 3 Graph of the relationship between the concentration of seasoning extracts with inhibition of conidia germination of *Aspergillus* spp.

The higher the concentration of seasoning extract used, the higher the inhibitory power of the germination of *Aspergillus* spp. The relationship between extract concentration and inhibitory ability to conidial germination followed the equation y = 78.3x + 65.33 with the coefficient of determination (R^2) = 0.8528 (**Figure 3**).

Inhibitory power of seasoning extracts against the density of conidia *Aspergillus* spp.

The results showed that seasoning extracts significantly ($p < 0.05$) inhibited the formation of conidia of 4 species of *Aspergillus* spp. The crude extract treatment with a concentration of 0.1 % was able to inhibit the formation of conidia of 4 types of molds of *Aspergillus* spp. which ranged from 72.9 - 78.3 % when compared to controls. The seasoning extracts treatment with a concentration of 0.1 % was able to inhibit the formation of conidia of *A. aculeatus, A. tubingensis, A. flavus,* and *A. niger* by 72.9, 73.9, 74.2 and 78.3 %, respectively when compared to controls (**Table 4**).

Table 4 Inhibition of seasoning extracts to the conidial density of *Aspergillus* spp.

Extract concentration	Conidia density (x 10^5 conidia/mL)				Percentage of inhibitory (%)			
(%)	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*
0	102.5 [a*]	101.6 [a]	97.7 [a]	103.4 [a]	-	-	-	-
0.1	26.4 [b]	26.5 [b]	21.2 [b]	26.9 [b]	74.2	72.9	78.3	73.9
0.2	14.6 [c]	13.7 [c]	11.5 [c]	14.2 [c]	85.7	86.5	88.2	86.3
0.3	5.7 [d]	5.9 [d]	5.6 [d]	6.1 [d]	94.4	94.2	94.3	94.1
0.4	1.3 [e]	1.7 [e]	1.2 [e]	1.9 [e]	98.7	98.3	98.8	98.2
0.5	0.0 [e]	0.0 [e]	0.0 [e]	0.0 [e]	100	100	100	100

*The average value followed by the same letter in the same column shows an insignificant difference based on Duncan's Multiple Range Test at 5 % level.

Meanwhile, the treatment of seasoning extracts with a concentration of 0.5 % can inhibit the formation of *A. flavus, A. aculeatus, A. niger,* and *A. tubingensis* by 100 % when compared to controls.

The results of this study in line with the results of research by [17] indicating that *Curcuma longa* oil at a concentration of 0.5 % was able to inhibit the formation of *A. flavus* conidia by 42.88 % when compared to controls. The ethanol extract of *Dittrichia viscose* at a concentration of 50 mg/mL was able to inhibit the formation of conidia of *A. fumigatus* strain CD 1435, *A. fumigatus* strain CD 1441, and *A. fumigatus* strain CD 1438 with a percentage of inhibitory power, respectively of 35.65;34.35;39.10 % compared to controls. Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) in fresh and dried form can inhibit the growth of the fungus microflora of *Aspergillus tamarii, Rhizopus stolonifer* and *Fusarium oxysporum* which can cause spoilage in smoked catfish [29].

The higher the concentration of seasoning extract used, the higher the inhibitory power of the conidial density of *Aspergillus* spp. The relationship between extract concentration and inhibitory ability to conidia density followed the equation y = 64.1x + 71.27 with the coefficient of determination (R^2) = 0.9016 (**Figure 4**).

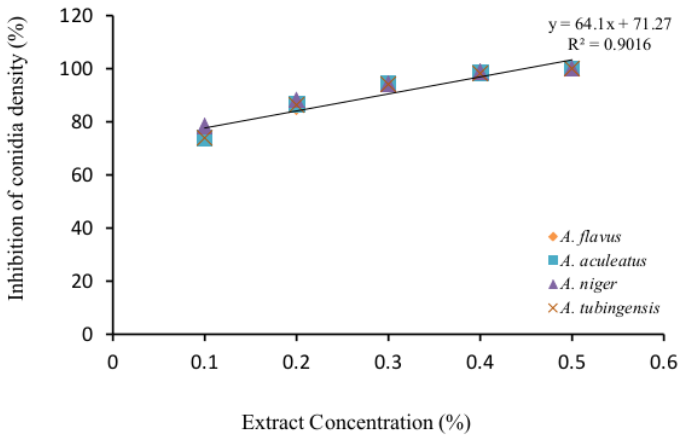

Figure 4 Graph of the relationship between the concentration of seasoning extracts and the inhibitory density of conidia *Aspergillus* spp.

Inhibition of seasoning extracts against *Aspergillus* spp.

The results showed that seasoning extracts significantly ($p < 0.05$) inhibited the dry weight of mycelia of 4 species of *Aspergillus* spp. The crude extract treatment with a concentration of 0.1 % was able to inhibit the dry weight of mycelia of 4 types of *Aspergillus* spp. which ranged from 80.73 - 90.99 % when compared to controls. Coarse extract treatment with a concentration of 0.1 % was able to inhibit the dry weight of mycelia *A. niger, A. aculeatus, A. flavus, and A. tubingensis*, respectively by 80.73, 82.25, 88.38 and 90.99 % when compared to controls (**Table 5**).

Meanwhile, the treatment of crude extract with a concentration of 0.5 % was able to inhibit the dry weight of mycelia *A. flavus, A. aculeatus, A. niger C,* and *A. tubingensis* by 100 % when compared to controls. The results showed that the spice extract contained alkaloids, terpenoids, flavonoids, and phenolics in which these compounds contributed to inhibiting the growth of molds of *Aspergillus* spp. so that it affects the dry weight of the mold mycelia.

Table 5 Inhibitory of seasoning extracts on the biomass of *Aspergillus* spp.

Extract concentration	Mold biomass (g)				Percentage of inhibitory (%)			
(%)	*A. flavus*	*A. aculeatus*	*A. niger*	*A. tubingensis*	*A. flavus*	*A. aculeatus*	*A. niger*	*A tubingensis*
0	1.086 [a*]	1.120 [a]	1.093 [a]	1.147 [a]	-	-	-	-
0.1	0.126 [b]	0.198 [b]	0.210 [b]	0.103 [b]	88.38	82.25	80.73	90.99
0.2	0.108 [c]	0.117 [c]	0.117 [c]	0.086 [c]	90.00	89.54	89.26	92.47
0.3	0.093 [d]	0.078 [d]	0.065 [d]	0.042 [d]	91.37	93.01	94.04	96.31
0.4	0.052 [e]	0.027 [e]	0.019 [e]	0.024 [e]	95.20	97.59	98.19	97.88
0.5	0.000 [f]	0.000 [f]	0.000 [f]	0.000 [f]	100	100	100	100

*The average value followed by the same letter in the same column shows an insignificant difference based on Duncan's Multiple Range Test at 5 % level.

The results of this study are in line with the research results of [30] that the treatment of coriander oil, fennel, and cumin with a concentration of 500 ppm was able to inhibit the dry weight of mycelia *A. flavus* strain ATCC 16872. Coriander can inhibit mold growth because it contains L-Linalool which can inhibit the hypha degeneration of mold. Sharma and Tripathi [28] reported that *C. sinensis* oil at a concentration of 1.5, 2 and 2.5 µg/mL were able inhibit the dry weight of *A. niger* by 91.70, 95.77 and 100 % when compared to controls. El-Desouky *et al*. [31] reported that the extract of fenugreek ethanol at a concentration of 1 % was able to inhibit the dry weight of *A. flavus* and *A. parasiticus* by 14.1 and 37.5 % when compared to controls. Gemeda *et al*. [19] reported that *Cymbopogon martini* extract at a concentration of 0.75 µl/mL was able to inhibit the dry weight of mycelia of *A. flavus* strain ATCC 13697 and *A. niger* strain ATCC 10535 inhibit at 71.18 and 91.85 % when compared with controls.

The higher the concentration of seasoning extract used, the higher the inhibitory power of the biomass of *Aspergillus* spp. The relationship between extract concentration and inhibitory ability against biomass followed the equation $y = 23.43x + 88.501$ with the coefficient of determination (R^2) = 0.9788 (**Figure 5**).

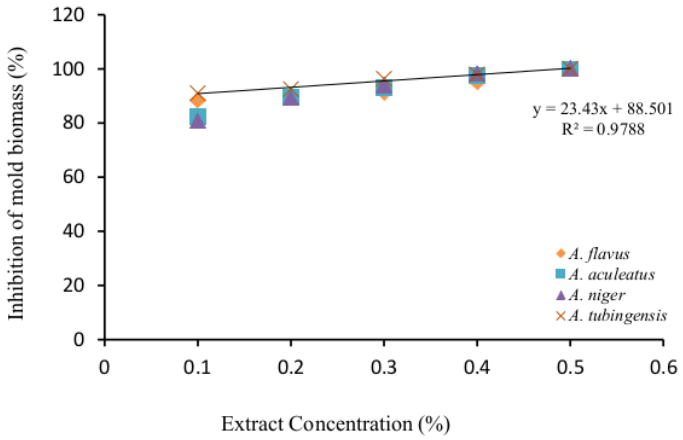

Figure 5 The graph of the relationship between the concentration of seasoning extracts and the inhibition of biomass of *Aspergillus* spp.

Observation results of *Aspergillus niger* hyphae using a scanning electron microscope (SEM)

The observation of *A. niger* molds hyphae by using SEM showed that *A. niger* molds hyphae showed differences between hyphae treated with spice extract treatment with control (**Figure 6**). The molds hyphae which were treated with spices extracts experienced lysis with a concentration of 0.1 %. It was shrinking, contracting, and hyphae damage, while the control treatment of molds hyphae remained intact and perfect.

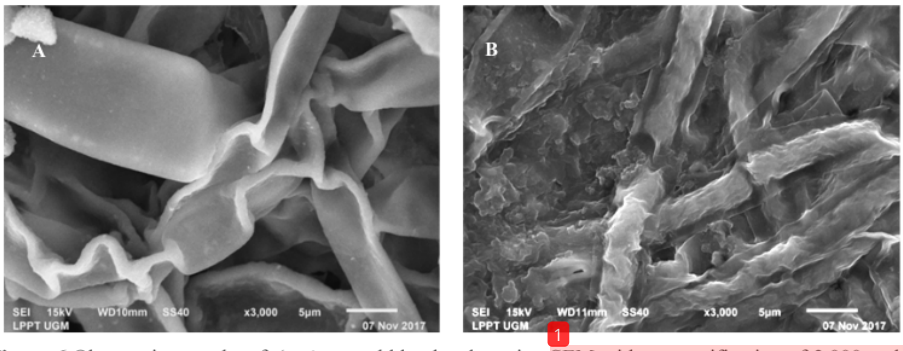

Figure 6 Observation results of *A. niger* mold hyphae by using SEM with a magnification of 3,000 and a 5 µm bar. A. hypha molds *A. niger* on control; B. hypha molds of *A. niger* which was given a seasoning extracts treatment of 0.1 % (w/v).

Phytochemical test results showed that the crude extracts of spices contained secondary metabolites such as alkaloids, terpenoids, flavonoids, and phenolics and no steroid and saponin compounds were found. These compounds are thought to contribute to anticipator activity. Alkaloids are compounds that have antimicrobial activity, namely inhibiting the enzyme esterase, DNA polymerase, RNA polymerase, and inhibiting cell respiration. The active compounds inhibit mold growth by disrupting cell membranes, inhibiting the formation of cell walls or damaging cell walls, changing cell membrane permeability that causes leakage of nutrients in cells, denaturing cell membrane proteins, damaging the metabolic system in cells by inhibiting the work of enzymes intracellular, and interfering with gene expression pathways [32].

Conclusions

The composition of garlic, coriander, kaemfiera, galangal, ginger, vinegar, and salt spices at 0.5 % extract concentration has inhibition zone diameter of minimum inhibitory concentration (MIC) in *Aspergillus aculeatus* of 0.8 mm, in *Aspergillus niger* of 1.2 mm, while in *Aspergillus flavus* and *Aspergillus tubingensis* of 1 mm. Seasoning extract at a concentration of 0.5 % is also able to inhibit up to 100 % of the growth of the colony, conidial germination, conidial density, and biomass of *A. flavus, A. aculeatus, A. niger,* and *A. tubingensis* that contaminate the sardine fishes. Alkaloids, terpenoids, flavonoids, and phenolic compounds contained in the spice extract inhibit the synthesis of proteins and enzymes needed in cell formation.

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