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# The ability of garlic extract and Kaempferia galangal inhibit Aspergillus sp. isolated from Sardine fish Pedetan

*by* Ni Made Ayu Suardani Singapurwa

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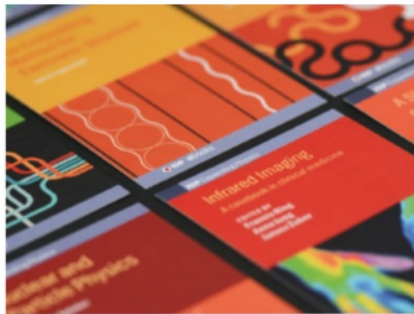
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## The ability of garlic extract and *Kaempferia galangal* inhibit *Aspergillus* sp. isolated from Sardine fish *Pedetan*

N M A S Singapurwa\*, I P Candra and I N Rudianta

Department of Food Science and Technology, Faculty of Agriculture,  
Warmadewa University, Terompong Street No. 24 Tanjung Bungkak, Denpasar, Bali,  
Indonesia

\*a.suardani@gmail.com

**Abstract.** *Pedetan* is one of the food products of the traditional Balinese fish Sardine which is processed by the community in the Jembrana Regency of Bali Province. This study aims to determine the antimicrobial activity of garlic and *kaempferia galangal* in inhibiting the growth of *Aspergillus* sp. which can contaminate the sardine fish *Pedetan*. The study was conducted by isolating *Aspergillus* sp. from *Pedetan* and testing the inhibitory power of garlic and *Kaempferia galangal*. The results showed that the extract of garlic and *Kaempferia galangal* can inhibit the growth of *Aspergillus* sp. From the results of SEM observations, it was found that garlic and galangal were able to damage the Mycelia *Aspergillus* sp. compared to control treatment. Garlic extract and *kaempferia galangal* can inhibit the growth of *Aspergillus* sp. because it contains bioactive compounds that are antimicrobial.

### 1. Introduction

Dry fish can be damaged during storage and during distribution in marketing. Damage to dry fish can occur due to microorganisms, such as bacteria and fungi, and damage due to enzymes, both enzymes derived from fish and from bacteria. Spices used in the process of making *pedetan* sardine fish can be used as one of the inhibitors of damage to dried fish products [1]. Garlic and *Kaempferia galangal* spices are used in the process of making *pedetan*.

Garlic contains Allicin active compounds that have anti-microbial activity [2]. As an antimicrobial, garlic can inhibit the growth of *Salmonella enteritidis* and *Staphylococcus aureus* [3]. Allicin and organosulphur compounds can inhibit the growth of gram-positive bacteria and gram-negative bacteria such as *Staphylococcus*, *Salmonella*, *Vibrio*, *Mycobacterium*, *Proteus* sp, *Helicobacter pylori* and also as anti-parasite, antifungal, and antiviral [4,5]. The content of active substances in the form of flavonoids, tannins, cineol and saponins in the galangal rhizome (*Kaempferia galanga* L.) has antifungal properties that can inhibit the growth of fungi *Candida albicans* [6].

The diverse processing of *pedetan* in each village is a different characteristic of *pedetan* producing villages. This also affects the quality and safety of these foods. Therefore the products produced are not uniformly quantitative or qualitative [7], making it difficult to standardize [8]. Therefore it is necessary to develop traditional food processing with several improvements in processing. The application of seasoning utilization will be able to improve the quality and security of traditional *pedetan* food. Quality management and safety aspects of raw materials and products need to be studied for business development, and product marketing development.



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This study aims to analyse the ability of garlic and galangal in suppressing the growth of contaminants in sardine fish ponds in an effort to ensure quality and food safety for consumers. This research is expected to be able to provide food safety information for sardine fish for consumers. This research will be very useful for producers engaged in traditional food processing, especially the sardine fish maker in an effort to provide a sense of comfort and safety for consumers.



Figure 1. Pedetan of sardine fish.

## 2. Materials and methods

This research was conducted at the Faculty of Agriculture Laboratory, Warmadewa University, Biopesticide Laboratory, Faculty of Agriculture, Udayana University, and at the Integrated Research and Testing Laboratory of Gajah Mada University Yogyakarta to see fungal hypha structures which are inhibited by a combination of garlic extract and galangal under the electron microscope.

Observation of the structure of hyphae of *Aspergillus* sp. with Scanning Electron Microscope. Testing the effect of garlic extract and galangal on *Aspergillus* sp. mold structure was done by adding 200  $\mu$ l extract of 10% concentration into Petri dishes and adding liquid PDA media (temperature 45°C), then Petri dishes were shaken horizontally so the extracts and PDA media were mixed equally. Mushroom colony pieces taken with a 5 mm cork diameter were placed in the middle of the PDA media on a Petri dish and repeated three times. Petri dishes were incubated at 25°C for 3 days. Mushroom colonies on the edge of the colony were cut to size 3 mm and 1 mm, the next step was the sample preparation process for SEM (Scanning Electron Microscope) analysis.

Mushroom colonies of 3 mm in size were then fixed with a solution of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C for 4 hours and after that, they were allowed to stand at room temperature (25°C) for 1 hour. The fixed sample was washed with sodium cacodylate buffer (pH 7.2) and continued fixation with 1% osmium tetroxide in 0.1 M cacodylate buffer and left at room temperature for 5 hours. After that the sample was washed using distilled water and then dehydrated using serial ethyl alcohol (40%, 60%, 80%, 99.5%, and 100%). After the dehydration process, the sample is cut using the freeze cutting device (TF-2, Eiko Japan). The sample was then given a solution of t-butyl alcohol and placed in a vacuum freezer drying device (ID-2, Eiko, Japan). The dried samples are placed in a special place and coated with osmium tetroxide (OPC 60A, Filgen, Japan) and Platinum (JUC-5000, JEOL, Japan). The coated sample was observed for SEM using JSM-6701F, JEOL, Japan with an acceleration voltage of 5 kV [9].

## 3. Results and discussion

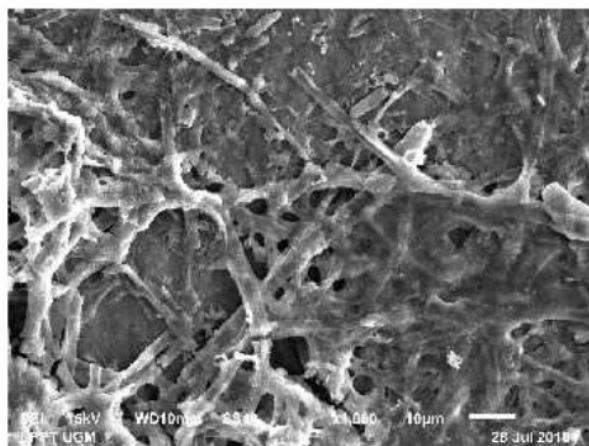
The observation results of a hypha of mold *Aspergillus* sp. using Scanning Electron Microscope (SEM) with a magnification of 3,000 and a bar of 5  $\mu$ m showed that hypha of mold *Aspergillus* sp. seen lysis after being given garlic and galangal extract with a concentration of 0.1% (Figure 2,3,4). Hifa molds treated with spice extracts were lysed after being given a 0.1% spice extract, which



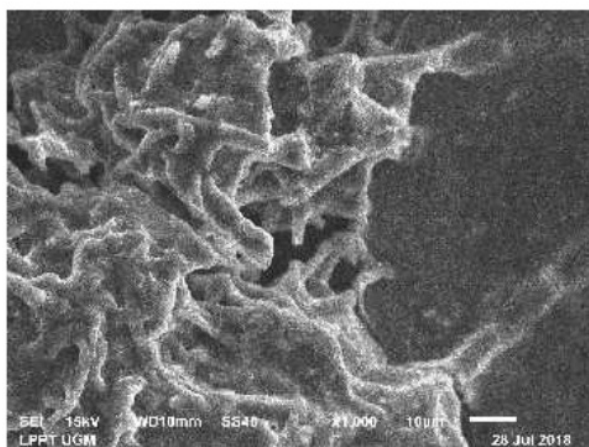
appeared to shrink, shrink and hyphae damage, whereas in hyphae control the mold remained intact and perfect.

The making of sardine fish sprouts using garlic spices can inhibit the growth of fungi with an inhibition zone of 21.15 mm and kaemfiera can inhibit the inhibition zone by 25.45 mm. Garlic and kaemfiera can inhibit fungal growth because they contain bioactive compounds that can be antimicrobial [10].

Garlic and galangal can affect the permeability of mold and bacterial cell membranes. If the permeability of the cell membrane is disrupted it will cause cytoplasmic leakage which results in lysis of the cells so that the cell dies [11]. Bioactive compounds can affect the growth of mold colonies, hyphae will shrink because of damage to cells and lysis, cytoplasm reduction and cell wall damage. The mechanism of anticancer activity from an essential oil is by damaging the cell wall and lysis of the cytoplasm which causes the death of mycelium and affects the growth of mold [12]. The lipophilic nature of essential oils will penetrate the plasma membrane which causes cell membrane damage [13].



**Figure 2.** Hifa mold of *Aspergillus* sp. on the treatment of 0.1% garlic extract.



**Figure 3.** Hifa mold *Aspergillus* sp. on the treatment of 0.1% galangal extract.



**Figure 4.** Hifa mold of *Aspergillus* sp. on the control treatment.

#### 4. Conclusion

From the results of observations of SEM (*Scanning Electron Microscope*) with a magnification of 3,000 and a bar of 5  $\mu\text{m}$ . it was found that garlic and galangal were able to damage the mycelia of *Aspergillus* sp. compared to control treatment.

#### Acknowledgments

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