Preparation and processing of garlic extract and its further application on anti-fungal activity

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Abstract
The anthracnose disease is brought on by the saprophyte fungus Colletotrichum musae (*C. musae*), which is prevalent in banana plantations. This study aimed to determine the effects of the garlic extraction process on the anti-fungal activity of *C. musae*. The concentration of garlic extract was studied at 20, 15, 10, and 5 ppm and tested the lowest concentration of garlic extract against anthracnose inhibition. Using absolute ethanol as a solvent was the best way to extract the substance from the banana. When testing the fungus with two methods, i.e., 1) the Agar Disc Diffusion method, measured the growth of mycelium at 12, 10, 10, and 9 mm, 2) The Dilution Susceptibility Test method, using concentrations 0, 5, 10, and 15, mean mycelium growth at seven days was 68.67+3.06, 31.00+3.00, 26.33+0.58, and 22.67+0.58 mm, respectively. The finding revealed that garlic extract could inhibit the fungus growth. The lowest concentrations were 10 and 15 ppm with no difference in inhibition, with mean values of 26.33+ 0.58 and 22.67+0.58 mm. The concentration at 10 ppm was the lowest concentration that could inhibit fungus in bananas by 75% compared to untreated banana hands.

Keywords: Anthracnose disease, bananas, garlic extract, anti-fungal activity

Thailand is located on the strip near the equator, causing hot weather and high humidity, which is suitable for producing agricultural products. Thailand is one of the world's top exporters of agricultural products, especially bananas which can be made in large numbers and are popular domestically and internationally. Bananas can be used to cook sweet and savory dishes. Additionally, cultivated bananas can be processed into products such as sun-dried bananas, sugar-boil banana puree, fried bananas, bananas in coconut milk, etc. The severe problems of banana were spoilage after four days of harvest caused by anthracnose. Anthracnose is caused by C. musae, a saprophyte fungus living in banana plots. Its spores can survive for long periods under high humidity and temperature conditions. The spores of this fungus can spread through the air and land on stalks of bananas (Jones, 2000) and can also be infected by pruning during processing. Anthracnose occurs on the terminal of the banana's hand and fruit. When bananas are infected, the fungus infests the banana's hands through the wounds, resulting in brown-to-black coloration and progression to slow rot. White fibers are formed around the wound. The banana fruit will have small, juicy brown spots on the skin of the banana peel and expand to destroy the skin of the banana peel. Inside the skin of the banana's fruit is a brown-black ulcer of varying size and shape. The most popular method for inhibiting this fungus is the use of chemicals such as Thiabendazole, Benomyl, Imazalil, Fluconazole, Itraconazole, and Amphotericin B (Muñoz, 2002). However, these chemicals may remain in the fruit, harm consumers, or spread to the environment. Due to the driving force of the organic market being to avoid the use of chemicals, the use of natural anti-fungal agents, therefore, becomes increasingly essential nowadays.

Garlic (Allium Sativum Linn) is a

plant with a robust and unique smell from sulfur as an essential component. It also contains allicin, an inhibitor of bacterial and fungal growth. Some studies investigated the substances in garlic that could inhibit fungal growth. In 1980, The Faculty of Medicine, Chiang Mai University experimented by crushing garlic to dissolve in water and mixing them with a fungal culture medium. As a result, garlic juice could inhibit the growth of mold. In 1992, garlic substances were tested to inhibit fungi by soaking cotton wool with juice from garlic and placing them in cultured fungi. It was found that the fungal growth slowed down and stopped. Most studies would extract garlic juice with solvents, such as ethanol, methanol, hexane, and ether.

The extraction method mentioned above yielded extracts with different anti-fungal activities. Most of these extracts had a robust inhibitory effect when used in high concentrations, with most studies conducted in vitro. However, the use of garlic extracts to inhibit pathogenic fungi in fruit has never been reported. Therefore, studying garlic extract to inhibit anthracnose in bananas would resolve the problem of postharvest management and shelf life extension. Additionally, the study of garlic extract to interfere with anthracnose in bananas was to increase the value of garlic as a medicinal plant to be used as a new product for further utilization. In this study, we studied the extraction of garlic extract to inhibit anthracnose. We extracted the substance from garlic that could deter C. musae from finding the appropriate dosage for inhibiting anthracnose and extending shelf life. Specifically, the study's objectives were to study the extraction method of garlic extract, the proper concentration of garlic extract to inhibit C. musae causing anthracnose in bananas, and the shelf life of bananas. In addition, this study would also obtain a guideline to analyze the utilization of garlic, an extension of the shelf life of bananas, and an alternative for the industrial use of Thai herbs as anti-fungal.

Material and Method

Garlic extract preparation

Garlic (80 g) was put in a large bowl, pounded finely, and added to a 600 ml beaker. Add Absolute ethanol (160 ml) was added and left at room temperature for 60 minutes. Then the solution was filtered with paper No. 4 into a 500 ml round bottom flask. The garlic extract was concentrated with a vacuum evaporator at room temperature and low pressure until no more solvent was evaporated. The garlic extract was stored in amber flasks at 4°C for further analysis. Then, record the weight of the garlic extract to calculate the percentage (Liu., 1989).

$$\text{Extract Percentage} = \frac{\text{Extract Weight}}{\text{Plant Sample Weight}} \times 100$$

After the garlic juice was filtered, the garlic residue was left on the filter paper. Then, the garlic residue was dried and weighed for further calculation in method of preparation of garlic extract (Sangvanich., 1984), as shown in Figure 1.

Weigh 80 g of garlic.

↓

Put the garlic in a large bowl and pound it finely.

↓

Scoop into a 600 ml beaker.

↓

Add Absolute Ethanol, volume 160 ml.

↓

Soak and leave at room temperature for 60 minutes.

↓

Strain the solution with filter paper No. 4 and put it in a brown 600 ml round bottom flask.

↓

A vacuum evaporator separated the extract at room temperature and low pressure until no solvent was evaporated.

↓

The extracts were stored in amber bottles, sealed at 4°C for further analysis.

↓

Garlic residue was dried and weighed to calculate the extract percentage.

Figure 1 Method of preparation of garlic extract (Somsiri and Sumitra, 1984)

Preparation of C. musae fungus

C. musae is an anthracnose fungal strain of bananas (courtesy of the fungi species from Mr. Thanat Amatayakul). The fungus was subcultured into potato dextrose agar (PDA) slant and incubated at room temperature for seven days before being stored at 4°C. To prepare the PDA culture medium, put 19.50XX g of the PDA culture medium into a 50 ml beaker. A 500 ml cylinder was used and measured 500 ml of distilled water. The solution was poured into a 1000 ml Duran flask, stirred well, and autoclaved at 121°C for 15 minutes. When the medium cooled, it was poured into medium agar dishes and waited for the agar to solidify. Then the Vertical Laminar Air Flow cabinet was opened. Before use, wipe with alcohol and turn on UV for 30 minutes before use. Cultivation of *C. musae* was carried out using a loop, sterilized, and then cut the agar surface of the fungi, measuring 3 mm in diameter. Placed the agar pieces in the middle of the agar medium in the agar medium dish, and then were incubated in an incubator at 30°C for seven days, the preparation process for *C. musae* fungus (Arneson, 1971) as shown in Figure 2.

Cultivatin of the C. *musae* fungus

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Cultivation of *C. musae* was carried out using loop, sterilized, and then cut the agar surface of the fungi, measuring 3 mm in diameter.

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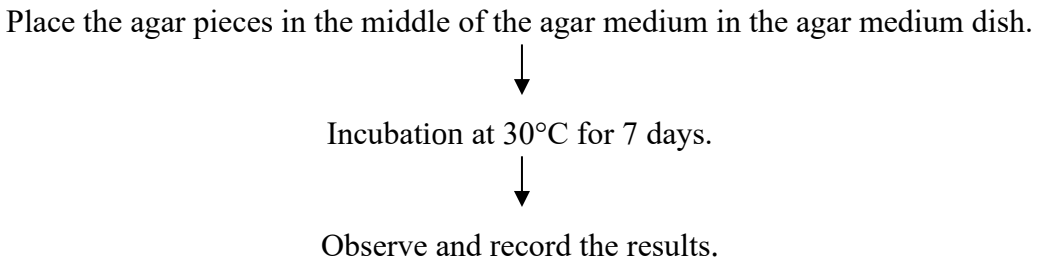


Figure 2 Preparation process for C. *musae* fungus (Arneson, 1972)

Preparation of garlic extracts at various concentrations

Preparation of garlic extracts at various concentrations

The garlic extract was serially diluted into 20, 15, 10, and 5 ppm. Distilled water was used as a control sample by adjusting the extract concentration volume, as shown in Table 1

Table 1 Volumetric adjustment of garlic extract concentration

Desired concentration (ppm)	Desired volume (ml)	Used concentration (ppm)	Concentrated volume used (ml)	Adjust volume with solution (ml)
5	50	100	2.5	Distilled Water
10	50	100	5	
15	50	100	7.5	
20	50	100	10	

Preparation of garlic and processing of garlic extract

Garlic extract is typically prepared by crushing or pressing garlic cloves and then extracting the active compounds using solvents or other methods. There are several methods for garlic extract preparation, including steam distillation, Soxhlet extraction, maceration, and ultrasonic extraction.

Steam distillation involves passing steam through the crushed garlic cloves, which then carries the volatile compounds into a cooling system where they condense into a liquid form. This method is commonly used for obtaining garlic essential oil, which is high in allicin and other volatile compounds.

Soxhlet extraction involves repeatedly extracting the crushed garlic cloves with solvents, such as ethanol or hexane, to isolate the active compounds. The solvent is evaporated to yield a concentrated garlic extract.

Maceration involves soaking crushed garlic cloves in a solvent for a period of time to allow the active compounds to diffuse into the solvent. The solvent is then evaporated to yield a concentrated garlic extract.

Ultrasonic extraction involves using high-frequency sound waves to break down the cell walls of the garlic cloves, releasing the active compounds into a solvent. This method is becoming increasingly popular due to its ability to produce high yields of garlic extract in a short amount of time.

After extraction, the garlic extract may undergo further processing, such as filtration, purification, or concentration, to

isolate and purify the active compounds. The resulting garlic extract may be in liquid or powder form, depending on the desired application.

In summary, garlic extract is prepared by crushing or pressing garlic cloves and then extracting the active compounds using various methods, such as steam distillation, Soxhlet extraction, maceration, or ultrasonic extraction. The resulting extract may undergo further processing to isolate and purify the active compounds.

Fungal inhibition efficacy tests

Agar disc diffusion method

The 7-day-old *C. musae* cultures were tested in a vertical laminar airflow cabinet. The cabinet was cleaned with alcohol and UV light for 30 minutes before use. After that, the loop was sterilized and cut into the agar surface of *C. musae*, measuring 3 mm in diameter. The agar slices of *C. musae* were placed in the center of the agar dish. The mycelial side of the fungus was turned upside down to touch the plate's agar medium's surface and incubated at 30°C for 2 days to allow *C. musae* to grow to a certain extent. A sterile No. 1 filter paper disc with a diameter of 5 mm was soaked with each concentration of the extracts and left for 20 s. The paper disc was placed on the surface of PDA about 2 cm away from the growing *C. musae* fungus for 2 days. The paper disc was pressed to stick to the food surface all over the disc. Next, two paper discs per 2 concentration levels were put on an agar plate, incubated at 30°C for 120 hrs., and repeated 3 times. The paper disc was soaked with distilled water as a control. The results were recorded by observing the mycelium growth compared to the control sample. The distance from the paper disc to the fungal border was measured in millimeters (mm). If the mycelial growth was less than that of the control sample, *C. musae* was inhibited.

Dilution susceptibility test method

PDA agar medium (19.5 g) was poured into a 50 ml beaker, filled with 500 ml of distilled water, and ran into a 1000 ml Duran flask. The mixture was then autoclaved at 121 °C for 15 minutes, put 9 ml into 4 test tubes, and left until the temperature was reduced to 45-50 °C. 1 ml of each concentration (Table 3.1) was mixed into the prepared PDA medium using a Vortex mixture and poured into the agar dishes, repeated 3 times. At each experimental concentration, 10 ml of garlic extract medium was obtained. For comparison, 10 ml of PDA medium was added after pouring the medium and leaving for 1 day to dry the surface of the medium and then transferred to the fungi to be tested.

Determining the lowest concentration of garlic extract that inhibited C. musae fungus

The cultured C. musae fungus was grown on a PDA medium in an agar medium dish cultivated at about 30°C for 5 to 7 days. A loop with a diameter of 3 mm was used to sterilize the medium and left growing. The agar pieces were then transferred onto a PDA medium containing garlic extract at 20,15, 10, and 5 ppm, respectively. The agar slices were placed in the center of the agar dish, with the mycelial side facing the media surface, and incubated at 30 °C for 7 days with a Complete Randomized Design (CRD). First, recorded the experimental results measured the growth of the growing fungal hyphae on the surface of the garlic extract medium at various concentrations by measuring the diameter of the growing colonies in the medium. Next, we compared the fungal growth in the control set and stopped recording the results. The obtained values were then used to calculate the growth

inhibition percentage from the following formula (Vudhivanich, 2010).

$$\text{Percentage of growth inhibition} = \frac{(A-B)}{A} \times 100$$

A = Mean diameter of fungal colonies on the comparative agar plates

B = Mean diameter of fungal colonies on the plate containing the extract

Testing on banana hand by immersion method

Brought the banana hand in the second phrase, which was the green skin, slightly yellowish, but greener. Then divide it into small hands, 3-5 per hand. The analysis result of item 3.9.2.2 showed that the lowest extract concentration was soaked with the whole banana hand at one concentration for 2 minutes. All concentrations must be controlled and left at room temperature (Figure 3). Recorded the results from imaging and disease incidence by counting the number of disease incidences and daily changes compared with the control. The obtained value was calculated for the percentage inhibition using the following formula (Vudhivanich, 2010).

$$\text{Percentage of disease inhibition} = \frac{(A-B)}{A} \times 100$$

A = Mean of control sample disease incidence

B = Mean of garlic extract disease incidence

Banana hands changed from slightly green to yellow

↓

Brought banana hands to be cut, 3-5 per hand

↓

Took the lowest extract concentration from the analysis results in item 3.9.2.2., then brought bananas to soak the exact for 2 minutes

↓

Incubation at room temperature

↓

Recorded the incidence by counting the number of disease incidences and daily changes.

Figure 3 Testing on the banana hand by immersion method

Results

The medium cultivar garlic was extracted with absolute ethanol solution at a ratio of 1:2 (80 g of garlic per 160 ml of absolute ethanol) at room temperature and immersed in absolute ethanol for 60 min. After evaporation by a rotary evaporator, the extract became a light yellow liquid with a weight of 18.1600 g or 22.70% of the extracted substance compared to the initial weight of garlic, from the calculation formula of Warunee (Yongsakulroj, 2004).

$$\text{Extract Percentage} = \frac{18.16}{80.00} \times 100 = 22.70\%$$

Figure 4 Garlic extract

The garlic extract was extracted with absolute ethanol for 60 min by the Agar

disc diffusion method, and C. musae growth was observed for 7 days. In agar plates with only fungal cultures, fungal growth was found to spread throughout the agar plates. The fungus would grow from the middle of the plate to the surrounding area, as shown in Figure 5.

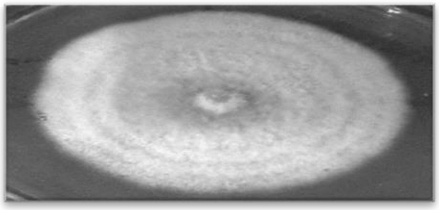
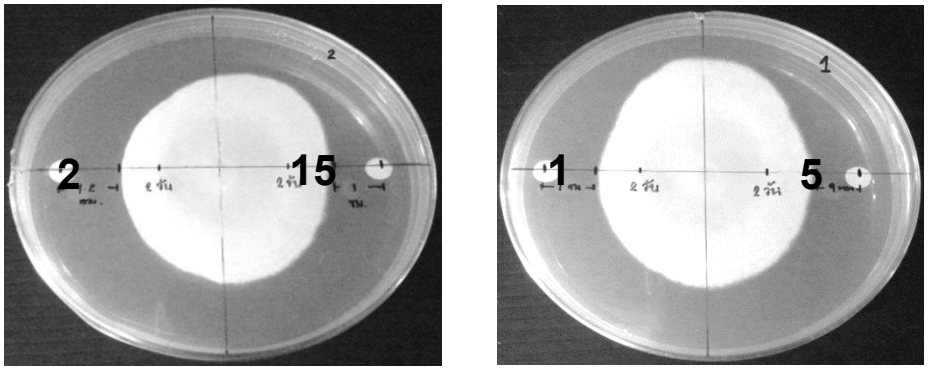


Figure 5 *C. musae* cultured on PDA medium for 7 days at 30°C.

From the result of C. musae fungus growth inhabitation by Agar Disc Diffusion method, Garlic extract extracted with absolute ethanol for 60 min showed that fungi (Figures 4.3 a. and b.) were able to grow less than those of the control plates (Figure 5) at the concentrations 20, 15, 10 and 5 ppm and 12, 10, 10, and 9 mm, respectively (Fig. 6a and b). It was shown that garlic extract extracted with absolute ethanol for 60 min could inhibit the growth of fungi.



a **b.**

Figure 6 the result of *C. musae* fungus growth inhabitation by garlic extract extracted with absolute ethanol. a.) Fungus and garlic extracts at concentrations of 20 and 15 ppm. b.) Fungus and garlic extracts at concentrations of 10 and 5 ppm.

The efficacy of garlic extract extracted with absolute ethanol in inhibiting colony growth of C. musae at 7 days of experimental age was examined. By measuring the mycelium growth of C. musae fungus, the fungus was grown on a PDA medium mixed with garlic extract by measuring the diameter of colonies growing horizontally and then compared with the bacteria in the control dishes (Table 2 and Figure 7).

Table 2 Comparison of the growth of C. musae fungus on PDA medium containing various concentrations of garlic extract.

Treatment	Mean colony diameter (mm)[1]
	7 days
0 ppm	68.67 ± 3.06^{a}
5 ppm	31.00 ± 3.00^{b}
10 ppm	26.33 ± 0.58^{c}
15 ppm	22.67 ± 0.58^{c}

Note: 1 = Mean from experimental sample 3 replicates, different vertical letters meant significantly different values ($p \leq 0.05$).

Table 2 shows the lowest concentration that could inhibit the growth of fungi in the PDA medium. The efficacy of garlic extract at concentrations of 0, 5, 10, and 15 ppm in inhibiting the growth of mycelium that causes anthracnose in bananas was compared with the control. It was found that the garlic extract at 10 and 15 ppm were not significantly different ($p > 0.05$). However, at concentrations of 5 ppm and concentrations of 10 and 15 ppm, there was a statistically significant difference ($p > 0.05$), and the best concentration that could inhibit C. musae fungus was 15 ppm (66.99%), followed by 10 ppm (61.66%) and 5 ppm (54.86%). The results showed that at concentrations of 10 and 15 ppm, there was no difference in inhibition ability. Therefore, 10 ppm was used to test the banana hand.

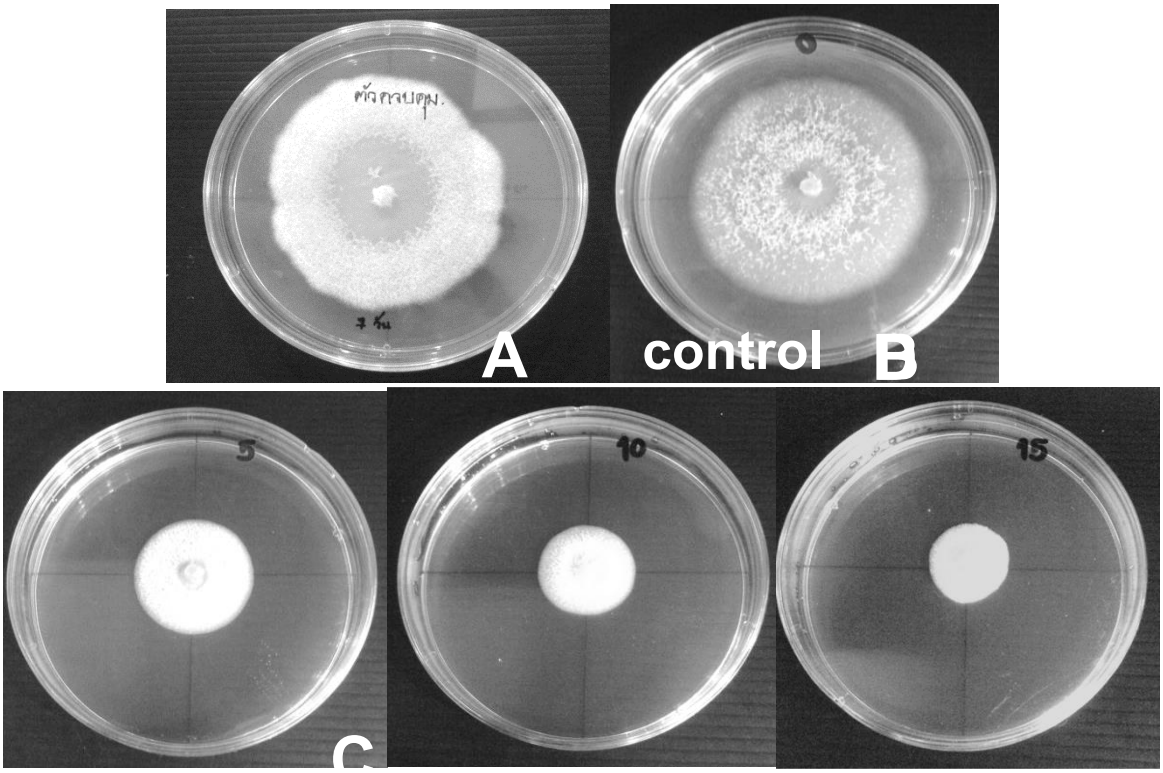


Figure 7 *C. musae* fungus growth in PDA culture medium mixed the garlic extract for 7 days. A: 0 ppm B: 5 ppm C: 10 ppm D: 15 ppm

Table 3 Efficacy test of garlic extract at a concentration of 10 ppm with banana hands for 7 days.

Day	Not Immersed in Distilled Water	Immersed in Distilled Water	Immersed in Garlic Extract
0			

1			
2			
3			
4			
5			
6			

Table 3 shows the changes in banana hands from the finding of the efficacy of garlic extract at a concentration of 10 ppm in inhibiting the growth of C. musae on banana hands compared with banana hands that were not soaked in distilled water and soaked in distilled water. Then recorded, the result as a picture as shown in Table 4.2. Garlic extract at 10 ppm effectively inhibited the growth of C. musae, the causative agent of anthracnose. Additionally, at the statistical level ($p > 0.05$), when comparing the extract immersed banana hands with the un-immersed banana hands, it was found that the extract immersed banana hands could inhibit the growth of C. musae (72.73%). When comparing the extract-immersed banana hands with distilled water-immersed bananas, it was found that the extract-immersed banana hands could inhibit the growth of C. musae (75.00%).

From garlic extraction with absolute ethanol solvent for 60 minutes, the extraction weight of garlic was 18.16 g, and the percentage of the extract was 22.70%. Garlic extract concentration was adjusted to 20, 15, 10, and 5 ppm. When bringing the extract to test the efficacy against C. musae, it was found that we could inhibit C. musae fungus

with the Agar Diffusion Test method. The finding revealed that all levels of concentration could effectively inhibit C. musae fungus. When comparing controlled samples with the Dilution Susceptibility Test method, it was found that the concentrations of 5, 10, and 15 ppm could inhibit the C. musae fungus at 54.86%, 61.66%, and 66.99%, respectively. When comparing the two methods, it was found that the Dilution Susceptibility Test method had significantly different inhibition values of different concentrations. At concentrations of 10 and 15 ppm, the ability to inhibit C. musae was not significantly different at the level ($p > 0.05$). For this reason, a lower concentration, 10 ppm, was used for banana hands, which could inhibit C. musae growth by 72.73% and 75% when compared with the un-immersed banana hands and distilled water-immersed banana hands.

Garlic extract can be prepared using different methods and solvents, resulting in extracts with varying properties and active compounds. Some of the most common types of garlic extract include:

Aged Garlic Extract (AGE): This extract is produced by soaking sliced or crushed garlic cloves in ethanol for a period of time, typically several months. During this time, the active compounds in garlic undergo chemical changes, resulting in a unique profile of compounds, including S-allylcysteine (SAC) and other sulfur-containing compounds. AGE has been shown to have antioxidant, anti-inflammatory, and cardiovascular benefits.

Allicin Extract: Allicin is a volatile compound produced when garlic is crushed or chopped. Allicin extract is typically prepared by steam distillation or vacuum distillation to isolate and concentrate the allicin. Allicin extract has potent antimicrobial and antifungal properties and has been studied for its potential as a natural alternative to antibiotics.

Garlic Oil: Garlic oil is produced by steam distillation of fresh garlic bulbs. It contains high levels of volatile compounds, including diallyl disulfide, diallyl trisulfide, and allyl mercaptan. Garlic oil has been studied for its antimicrobial and antioxidant properties.

Water-soluble Garlic Extract: This extract is produced by soaking garlic in water and then purifying the extract to remove impurities. It contains a range of sulfur-containing compounds, including alliin, which is converted to allicin when garlic is crushed or chopped. Water-soluble garlic extract has been shown to have antimicrobial, anti-inflammatory, and cardiovascular benefits.

Black Garlic Extract: Black garlic is made by heating whole garlic bulbs at low temperatures over a period of weeks, resulting in a sweet, caramelized garlic with a different profile of active compounds than fresh garlic. Black garlic extract contains high levels of S-allylcysteine (SAC) and has been studied for its antioxidant and anti-inflammatory properties.

Furthermore, garlic extract has been found to be effective against a range of fungal species and has been studied both in vitro and in vivo for its anti-fungal properties. However, as with any natural remedy, there may be potential side effects and interactions with certain medications, so it is important to use garlic extract under the guidance of a healthcare professional.

Meanwhile, Garlic extract has been extensively studied for its anti-fungal properties and has been found to be effective against a range of fungal species. Some of the most commonly studied fungi include Candida species, Aspergillus species, and dermatophytes. Studies have shown that the

active compounds in garlic extract, including allicin and other sulfur-containing compounds, have potent anti-fungal activity. These compounds work by disrupting the cell membranes and other key structures in the fungal cells, leading to their death or inhibition of growth.

Garlic extract has been found to be effective in both in vitro and in vivo studies. In vitro studies involve testing garlic extract in a laboratory setting, while in vivo studies involve testing the extract in living organisms, such as animals or humans. In vivo studies have shown that garlic extract can be effective in treating fungal infections of the skin, nails, and oral cavity.

While garlic extract is generally considered safe when consumed in moderation, there are some potential side effects that should be considered. These include gastrointestinal discomfort, such as bloating, gas, and diarrhea, as well as allergic reactions in some individuals. Garlic extract can also interact with certain medications, including blood thinners and some HIV medications, so it is important to consult with a healthcare provider before using garlic extract for any health purposes and type of garlic extract and its specific properties depend on the method of extraction, solvent used, and the compounds that are concentrated. Different types of garlic extract have been studied for a range of potential health benefits, including antimicrobial, antioxidant, anti-inflammatory, and cardiovascular effects.

Garlic Extract and Its Antifungal Applications : Garlic, or Allium sativum, has been used for centuries in traditional medicine for its various health benefits, including its anti-fungal properties. Garlic contains several sulfur-containing compounds, such as allicin, alliin, ajoene, and diallyl disulfide, which have been shown to exhibit anti-fungal activity. It is a concentrated form of these compounds that is obtained by crushing or pressing garlic bulbs and then extracting the active compounds using solvents or other methods. Garlic extract has been studied for its potential anti-fungal applications in various contexts, including in agriculture, food preservation, and medicine. It can be show in 3 parts as follow:

In agriculture, garlic extract has been used as a natural fungicide to control fungal infections in crops. Studies have shown that garlic extract can effectively inhibit the growth of several types of plant pathogenic fungi, including Fusarium oxysporum, Alternaria alternata, and Botrytis cinerea. Garlic extract has also been shown to enhance plant growth and increase crop yields.

In food preservation, garlic extract has been studied as a natural preservative to prevent fungal growth in food products. Studies have shown that garlic extract can effectively inhibit the growth of several types of food-borne fungi, including Aspergillus niger, Penicillium roqueforti, and Candida albicans. Garlic extract has also been shown to have antioxidant and antibacterial properties, which further contribute to its potential as a natural preservative.

In medicine, garlic extract has been studied for its potential anti-fungal properties in the treatment of various fungal infections. Studies have shown that garlic extract can effectively inhibit the growth of several types of human pathogenic fungi, including Candida albicans, Aspergillus fumigatus, and Trichophyton mentagrophytes. Garlic extract has also been shown to enhance the activity of conventional anti-fungal drugs, such as fluconazole and itraconazole, suggesting its potential as an adjunct therapy for fungal infections.

Overall, garlic extract shows promising potential as a natural anti-fungal agent with various applications in agriculture, food preservation, and medicine. However, further research is needed to fully understand its mechanism of action and to determine its optimal dosage and mode of administration for different contexts.

On the other hand, garlic extract is generally considered safe when consumed in moderate amounts. However, excessive consumption of garlic extract may lead to gastrointestinal symptoms, such as nausea, vomiting, and diarrhea. Garlic extract can also interact with certain medications, including blood thinners and some HIV medications, so it is important to consult with a healthcare provider before using garlic extract for any health purposes.

In addition, there have been some reports of skin irritation and allergic reactions in individuals who use garlic extract topically. It is important to do a patch test before applying garlic extract to the skin and to discontinue use if any irritation or allergic reaction occurs. Long-term using for garlic extract in high doses may also have some potential risks, including a possible increased risk of bleeding and a potential impact on liver function. However, more research is needed in this area to fully understand the long-term effects of garlic extract use.

Moreover, while garlic extract is generally considered safe when used in moderation, there are potential risks and long-term effects associated with its use. It is important to use garlic extract under the guidance of a healthcare professional and to be aware of any potential interactions with medications or other health conditions.

Discussion

The study was about extracting garlic extract to inhibit anthracnose in bananas. The garlic extraction process used a calculation formula of Watcharin (Liu., 1989) . It used the solution from the extraction of medium varieties of garlic with a 1:2 absolute ethanol solution (80 g of garlic per 160 ml of absolute ethanol) at room temperature. The garlic was immersed in absolute ethanol for 60 min and then evaporated by a rotary evaporator. The extract was a light yellow liquid with a weight of 18.1600 g, or 22.70% of the extract, compared to the initial weight of garlic. The extract was similar in quantity and characteristics to the study of Pornpana (naksing, 2007), who studied the fungicide effect of ethanol extract from pomegranate peel on Colletotrichum gloeosporioides, the causative agent of capsicum anthracnose disease. He used the absolute ethanol extraction method with a ratio of 1:2. The resulting extract was a light yellow liquid, and the weight was 17.1580 g or 21.50%. (Bhasabutra, 1997) Tharntip used the Dilution Susceptibility Test method extracted with an absolute ethanol solution to test 61 plant extracts against the growth of Colletotrichum musae, the causative agent of mango anthracnose, in the laboratory. It was found that the garlic extract by extraction method using solvent could prevent mango anthracnose disease well. Moreover, Khet studied the extraction method of allicin from garlic and found immersion in absolute ethanol for 60 mins and evaporation by a rotary evaporator would make a light-yellow liquid extract that was effective in inhibiting the growth of various microorganisms. Therefore, the study result revealed that the absolute ethanol extraction method made the highest number of light yellow liquid extracts, which were expected to be the most effective in inhibiting microbial growth.

At concentrations of 10 and 15 ppm, the C. musae inhibitory activity was not

significantly different at the level. Therefore, a lower concentration, 10 ppm, was applied to the banana hand, which inhibited the growth of C. musae by 72.73% and 75% compared to the unsoaked banana hand and the soaked banana comb with distilled water, respectively. The test result was consistent with the study of Tharntip (Bhasabutra, 1997) who studied using plant extracts to prevent anthracnose disease. It was found that the Dilution Susceptibility Test method was a method that provided extracts with more efficacy in inhibiting infection than other methods. Furthermore, Wanatnan studied the inhibitory effect of the long pepper extract on Colletotrichum gloeosporioides, the causative agent of mango anthracnose. (samitharporn, 2004) Siriwan studied the control of anthracnose and fruit pole rot in Nam Dok Mai mangoes by using plant extracts. The study found that the Dilution Susceptibility Test and the concentration of the extract concentration level of 10 ppm was the lowest level that could inhibit the growth of microorganisms.

Additionally, it was consistent with the study of Jutarat, who studied the influence of some medicinal plants on the growth of Pythium sp., the causative agent of cantaloupe root rot. It was found that the immersing some medicinal plants in the absolute ethanol solution, the extract became a pale yellow liquid. Therefore, the dilution susceptibility test was used to determine the lowest concentration to inhibit the growth of Pythium sp. fungi using the lowest concentration of 11 ppm. Therefore, using absolute ethanol as a solvent to extract the active substances in garlic would get a light yellow liquid extract. On the other hand, using the dilution susceptibility test with distilled water as a diluent to find the lowest concentration was effective and able to find the least amount of extract used to inhibit the growth of microorganisms.

Certainly, there is a significant body of literature on the use of garlic extracts for their anti-fungal properties. Garlic extracts have been shown to have a wide range of effects on various fungal species, including Candida, Aspergillus, and dermatophytes. One study published in the Journal of Antimicrobial Chemotherapy demonstrated that allicin, a compound found in garlic, had a strong anti-fungal effect on Candida species. The study found that allicin was able to damage the fungal cell membrane, leading to cell death.

The another study published in the International Journal of Dermatology investigated the use of a garlic extract gel for the treatment of fungal infections of the skin. The study found that the garlic extract gel was effective in reducing the severity of fungal infections and improving symptoms such as itching and scaling. Several other studies have also investigated the anti-fungal properties of garlic extracts, including their potential use in the treatment of oral thrush, a fungal infection of the mouth and throat.

While many studies have shown promising results, it is important to note that more research is needed to fully understand the mechanisms of action of garlic extracts on fungal cells and to determine the optimal dosages and formulations for their use. Additionally, as with any natural remedy, it is important to use garlic extracts under the guidance of a healthcare professional and to be aware of any potential interactions with medications or other health conditions.

In conclusion, this study found that the compounds in garlic were interesting substances as garlic extracts could inhibit the growth of fungi. It could hinder the growth of *C. musae*, and also included many properties that could be used as food additives to extend the shelf life of various food products.

Acknowledgments

This research was supported by Rajamangala University of Technology Phra Nakhon of Foundation. In addition, we thank our colleagues from Home Economics Technology, who provided insight and expertise that greatly assisted the research, and we thank the Dean of Home Economics Technology for comments that greatly improved the manuscript.

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