

Antimicrobial Activity of Plant Extracts from Aloe Vera, Citrus Hystrix, Sabah Snake Grass and Zingiber Officinale against Pyricularia Oryzae that causes Rice Blast Disease in Paddy Plants

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Abstract. Rice blast disease, caused by the fungus known as *Pyricularia oryzae*, has become an important and serious disease of rice worldwide. Around 50% of production may be lost in a field moderately affected by infection and each year the fungus destroys rice, which is enough to feed an estimated 60 million people. Therefore, use of herbal plants offer an alternative for the management of plant diseases. Herbal plant like Aloe vera, Citrus hystrix, Sabah snake grass and Zingiber officinale extracts can be used for controlling disease of rice blast. In this study, these four herbal plants were used for evaluating antimicrobial activity against rice plant fungus *Pyricularia oryzae*, which causes rice blast disease.

1. Introduction

Among issues raised by farmers are the epidemics of new and emerging plant diseases, threatening the food security. This issue will give huge impacts and attention on understanding for controlling the pathogens. Thus rates of introduction of exotic pathogens have increased in recent years, driven largely by the altered patterns and increasing rates of travel and trade. Under the economic transformation programmed (ETP), the Malaysian government wants to ensure that food security objectives are achieved. There is a need to struggle for invading this problem especially in the agriculture-based countries [1-2]. In this problem, rice was selected because it is an important crop worldwide, over half of the world population relies on it for food, including Malaysia where all the Malaysian are eating a rice as their staple food in daily life [3]. Rice cultivation is found most widely in Asia. The demand for rice is expected to rise more higher as the world's population increases. Rice is the most major and number one crop in Malaysia. It is the main source of energy for the people, as it contains higher value of carbohydrate, which provides more than 300 calories per 100 g of rice. Besides that, rice is rich with nutrients other than that it has lower value in fat and cholesterol, it actually contain higher value in starch. It is also one among many foods that are very easy to digest [4]. However, there are many factors that make paddy rice production become slow and less productive nowadays. One of the main causative reasons is paddy disease [5]. Among the common diseases, rice



blast disease is caused by *P. oryzae* [6] and Rice tungro disease (RTD) [7], which is the most destructive disease in rice-growing countries worldwide. Around 50% of production may be lost in a field moderately affected by infection and each year fungus destroys rice enough to feed an estimated 60 million people [8]. Rice blast disease is one of the major factors causing losses in quality and quantity of rice [9] where *P. oryzae* fungus that attacks the paddy plant can be found in all the regions of rice-growing environments. The fungus can attack all part of the paddy plant at different growth stages, either on leaf, grain and also the on neck of the paddy. Plant disease will reduce the production and quality of food crops and also causing significant losses to farmers and has big impact towards the economy as it threatening the food security. Concerns regarding food safety and the environment have led to the reduced use of agrochemicals and the development of sustainable agriculture. In this context, the focus of biological control studies reflects the desire of several sectors to develop sustainable methods for plant disease control.

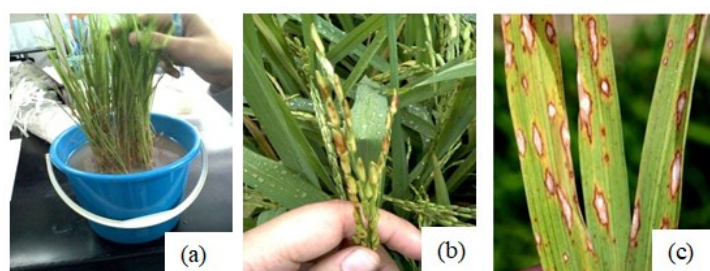


Figure 1. (a) Sample of the infected paddy plant by *P. oryzae* from MARDI; (b) Symptoms on grain of paddy plant; (c) Infection on paddy leaf.

Herbal medicinal plants such as *A. Vera*, *C. hystrix*, *Z. officinale* and Sabah snake grass have been proved in the previous study that the plant has antifungal properties. *A. vera* from Aloe family has long association with herbal medicine and Aloe gel is the most widely recognized herbal in the world today, as it contains a wide range of enzyme which provides antiseptic, anti-inflammatory and also antifungal benefits [10]. Anthraquinones are major substances found in aloe, and are known for their laxative, anti-microbial and anti-cancer activities [11]. *C. hystrix*, commonly known as kaffir lime, is a common tropical herb in the family Rutaceae found everywhere in Southeast Asia. *C. hystrix* is a thorny bush with aromatic leaves and dark green fruits with irregular bumpy surface. The valued parts of *C. hystrix* are the leaves and fruit peel which contain natural antioxidants and active compound that is important in fighting the diseases and cancer [12]. *Z. officinale* is belongs to the family of Zingiberaceae, a perennial herb with thick tuberous rhizomes. The gingerols is the most active compound found in ginger and showed that *Z. officinale* extracts have medicinal properties, and antimicrobial activity as reported [13] other than that as an antioxidant, anti-lipid, anti-diabetic, analgesic, and anti-tumor [14]. Sabah Snake Grass is suitable for plant growth in tropical weather such as Malaysia and Thailand. This plant also has antimicrobial activity similar to *A. vera* and suitable to use in this research. In Malaysia, the plant is popularly known as “Sabah snake grass” in English since it was found in Sabah of East Malaysia. The marginally curved stem supporting the leaves are look like the curve of an elephant’s trunk and therefore it is also named as Daun Belalai Gajah (elephant’s trunk) in Malay [15].



Figure 2. (a) *A. vera* leaf; (b) *Z. officinale* rhizomes; (c) *C. hystrix* leaf; (d) Sabah Snake Grass

2. Materials and Methods

2.1. Materials

Ethanol, Potato Dextrose Agar (PDA) media, Sodium Chloride, Tween-20 were purchased from Sigma-Aldrich. All analytical grade reagents were from Merck. All chemicals and reagents were stored and used as recommended by the supplier

2.2. Collection of infected paddy plant

The paddy plant which have been infected with *pyricularia grisea* are supplied by Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai . Then the fungus, *Pyricularia oryzae* was isolated for future analysis.

2.3. Preparation of *A. Vera* extraction

Firstly, the Aloe leaves are dried in the oven at 80°C for 48 hr. After that, the samples that have been dried are crushed into the small pieces using the blender until it became powdered. The solvent used is ethanol. For the preparation of ethanol, 20 g of the powder is soaked in 100 ml of the solvent and put on incubator shaker for 24 h. The mixed solution then is filtered and was kept in the refrigerator until further use.

2.4. Preparation of *Z. officinale* extraction

The rhizomes are washed, peeled and washed again in clean water. After washing, the rhizomes are cut into small pieces and were dried in the oven for 5 hours and 30 minutes. The gingers then are ground using an electric blender until it becomes powdered. 20 g of the ground material (*Z. officinale*) will be placed in a conical flask containing 100 ml of ethanol solvent and will be put on incubator shaker for 24 h. The mixed solution then is filtered and was kept in the refrigerator until further use.

2.5. Preparation of *C. hystrix* leaf extraction

The leaf of *C. hystrix* is washed with clean water for 3 times. After that, the leaves are cut into small pieces and dried it for a 30 seconds using microwave. After that, the leaves were machine using electric blender until it became powder. 20 g of the powder then are placed in conical flasks containing 100 ml of ethanol solvent and was put on incubator shaker for 24 h. Next, the mixed solution then is filtered and was kept in the refrigerator until further use.

2.6. Preparation of Sabah Snake Grass Extraction

For Sabah snake grass, the collected leaves were spread in a single layer on a tray and left at room temperature ($25 \pm 1^\circ\text{C}$) for 18 hours for withering process to obtain about 70% of moisture content. Withered leaves were grounded into relatively small particle size using a blender for 15 sec. The blended leaf will be soaked with ethanol and kept in incubator shaker to mix the solvent well for 48

hours. The extracts were filter using Whatman filter paper (No. 1) and then they were kept in refrigerated condition until use.

2.7. Preparation of Potato Dextrose Agar (PDA) media

PDA was prepared by dissolving 39 g of PDA with 1 L of distilled water in a scotch bottle. The bottle then is shaking for many times. After that, the solution inside the scotch bottle is autoclaved for 15 minutes. Then, the scotch bottle is taken out from the autoclave machine and placed it in the laminar flow and allows it to cooling down. Next, the PDA solution was poured into the empty petri dish and allowed it to solidify.

2.8. Preparation of microbial cultures

First step, the infected portion *P. oryzae* is cut into small pieces and sterilized. After that, rinsed it three times with sterile distilled water and was crushed using pestle and mortar, then it was transferred onto the surface of PDA agar. The second method is the infected grain paddy was taken, washed and dried and then transferred it on PDA agar. The fungus is incubated at 30°C for 7 to days. The mycelium growing out of the plant tissue is then subculture on new PDA media and incubated at 30°C for 7 to 10 days. The subculture process is repeated for three times to obtain the pure colony. The fungus then is identified based on the morphological and cultural characteristics based on the observation under the microscope.



Figure 3. Process of preparation of microbial culture

2.9. Screening of antimicrobial activity

Screenings of antimicrobial activity are performed by standard disc diffusion method. The disc paper is used for evaluating the antimicrobial activity. The discs are prepared from Whatman No. 1 filter paper. The size of the disc is 6 mm. The discs are ensured to sterilized and autoclaved. After the sterilization the moisture discs are dried until further use. Then the three plants extracts discs and control discs are prepared.

2.10. Antimicrobial activity of *A. Vera*, *C. hystrix*, *Z. officinale* and Sabah Snake Grass

The antimicrobial activity is carried out by using disc diffusion technique. The fungal cultures are put on the PDA agar medium that has been prepared well and was grow for 7 days at the centre of the media. The disc is also put around the fungus and the three different plant extracts with three different concentrations (10, 20 and 30 mg/ml) was pipetted on it. The plates then are incubated at 30 °C for 7 days and the clear zone developments was closely monitor.

2.11. Determination of Minimal Inhibition Concentration (MIC)

The lowest concentration of the extractions where the effect is smaller is considered as the minimal inhibitory concentration.

2.12. Data Analysis

ANOVA analysis is used to test the significance different of the results between the three different of plants extracts and the differences in the concentrations.

3. Results and Discussions

3.1. Identification of *P. oryzae*

The measurement of mycelial growth and colony diameter were taken and growth pattern of *P. oryzae* on PDA was observed. The margin of colony of *P. oryzae* was like white, round, cottony and with fluffy mycelium surface shown on the PDA. Morphology of spores under microscope was also observed. Another main characteristic of *P. oryzae* is it pear-shaped of its conidia when observed under the microscope. The isolated fungus from paddy plant in this research, which was grown on PDA has a radial growth of approximately 40 mm to 50 mm after 8 days of growth as shown in the figure 4 below.



Figure 4. Fungus *P. oryzae* being cultured on PDA

The figure 5 below shows the result from the culture media, under 400X magnification (a) the microscopic morphology of *P. oryzae* (b) the colony morphology on PDA and (c) the conidia from the culture.

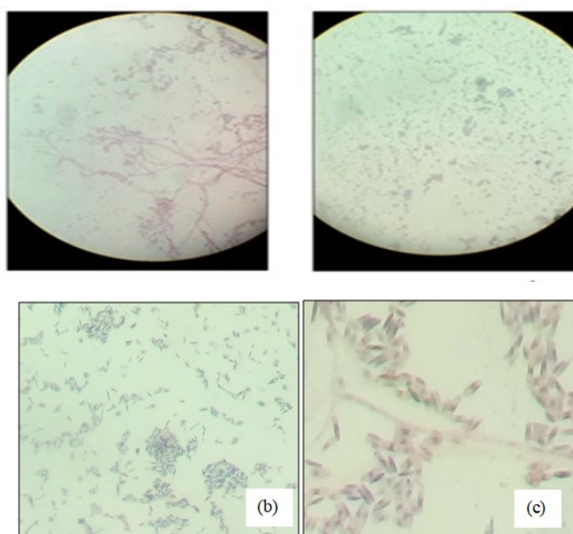


Figure 5. (a) The microscopic morphology of *P. oryzae* obtained, (b) *P. oryzae* colony under microscope (c) Conidia from the culture (pear-shaped)

After that, the step is continued with the process of subculture. Sub-culture was conducted at many times from each successive sub-culture before obtaining a completely pure culture. The figure 6 below showed the successive of sub-cultured fungus of *P. oryzae*.



Figure 6. Successive sub-cultured of the fungus

3.2. *In-vitro* antimicrobial activity of *A. Vera*, *C. hystrix*, *Z. officinale* and Sabah Snake Grass extraction against *P. oryzae* fungus

The method used to test the antifungal activity towards the fungus *P. oryzae* was by using disc diffusion method. The zone of inhibition that was formed is recorded. Table 1 and figure 7 shows the response of the fungus towards the *A. vera*, *C. hystrix*, *S. snake grass* and *Z. officinale* plants extraction at three different concentrations which are 10 mg/ml, 20 mg/ml and 30 mg/ml in table and bar graph form. All the activities testing were repeated three times or triplicated. Figure 5 below showed the antifungal testing of the four plants extracts against the fungus.

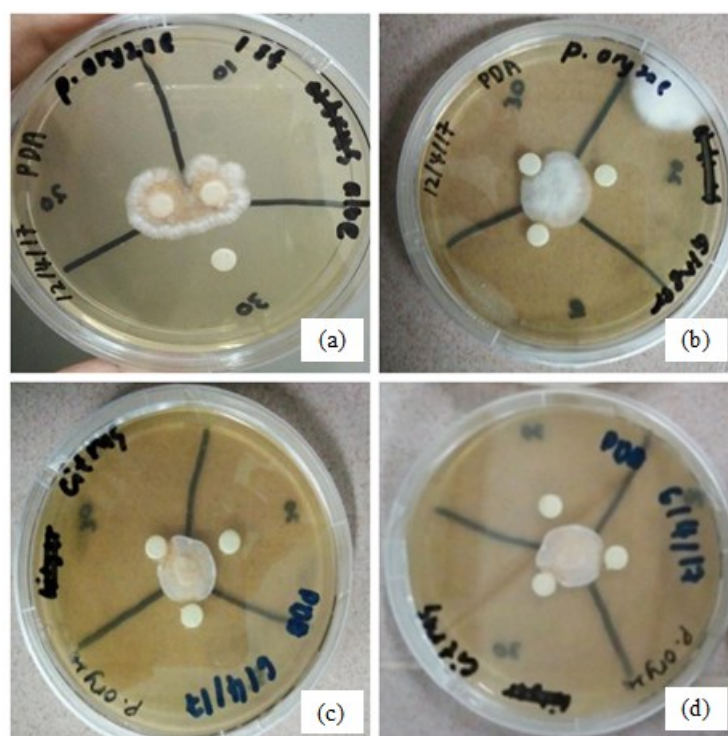
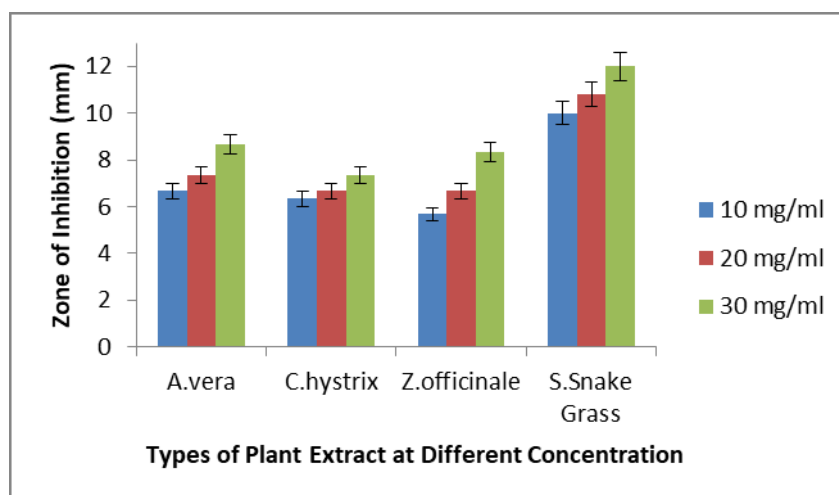


Figure 7. Antifungal testing (a) *A. Vera* (b) *Z. officinale* (c) *C. hystrix* (d) Sabah Snake Grass

Table 1. Zone of Inhibition on the fungus

Plant Extracts	Zone of Inhibitions (mm)		
	Concentration (mg/ml)		
	10 mg/ml	20 mg/ml	30 mg/ml
A. vera	6.67	7.33	8.67
C. hystrix	6.33	6.67	7.33
Z.officinale	5.67	6.67	8.33
S. Snake Grass	10	10.8	12

The results obtained indicate that the concentrations of the tested plant extracts against *P. oryzae* had a positive effect in inhibiting the mycelia growth. From the results, Sabah snake grass extraction has the highest inhibition value at concentration 30 mg/ml, followed by A. Vera, Z. officinale and C. hystrix extraction. The higher the concentration, the bigger the size of the inhibition should be. The lowest inhibition zone recorded for four types of plant extracts was at the concentration of 10 mg/ml whereas the Sabah snake grass extract has diameter of 10 mm, A. vera extract has diameter of 6.67 mm and C. hystrix has diameter of 6.33 mm. As for Z. officinale, the lowest inhibition zone was recorded at concentration of 20 mg/ml which is 6.67 mm. The negative control used is distilled water. There is no inhibition zones for the negative control that showing in distilled water; this proves that the distilled water is not the antibacterial agent. For the positive control, which is DMSO, there is inhibition zone shown which concluded that DMSO can be used as an antifungal agent. From ANOVA analysis, there is no significant difference between the three concentrations and the inhibition zones of the four types of plant extractions as the value of F-calculated is smaller than F-critical value and p-value is bigger than 0.05. Different inhibition studies have been demonstrated in the past against different pathogens [16-19]. The current study is also shown to have inhibition against rice blast disease causing pathogen, similar to the past studies and complementing as a new addition.

**Figure 8.** Bar chart of the Inhibition zone on the fungus for antifungal activity

4. Conclusion

In this study, the extraction of A.vera leaves, C. hystrix leaves, the rhizome of Z. officinale and Sabah snake grass proved that they have potential natural antimicrobial activity against the fungus of *P. oryzae* from the paddy plant that caused the rice blast diseases. The fungus of *P. oryzae* was successfully isolated from the paddy plant. Among all the parts from paddy plant, which are the neck

of the paddy, leaf and the grain that were taken and isolated, the grain part is the part that has been successfully isolated and been identified as *P. oryzae* fungus. The main characteristics are its white colony colour, fluffy cottony look on PDA and pear-shaped conidia is observed under the microscope. In this experiment, three concentrations (10, 20 and 30 mg/ml) of each ethanol plants extract (*A. vera*, *C. hystrix*, Sabah snake grass and *Z. officinale*) were used to inhibit the growth of causative fungus of rice blast disease on paddy plant, *P. oryzae* and get the minimum inhibitory concentration (MIC). The inhibition zones formed are recorded. Basically, the stronger the inhibitory activity of the plant extracts, the larger inhibition zone is formed. The lower concentration of crude extract tested, the small inhibition zone is shown. From the results, Sabah snake grass extraction has the highest inhibition value at concentration 30 mg/ml with 12 mm of the inhibition zone formed, followed by *A. vera* with 8.67 mm, *Z. officinale* with 8.33 mm and *C. hystrix* extraction with the diameter of the inhibition zone of 7.33 mm.

Acknowledgement

The authors wish to thank Universiti Malaysia Perlis for providing financial and technical support to conduct this research. We acknowledge the support from “Geran Penyelidikan Jangka Pendek” (9001-00552).

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