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Short Communication

Antimicrobial activity of Indonesian plant extracts against food borne microorganisms

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Abstract

Many pathogens such as fungi, yeast or bacteria commonly infect food supplies throughout post-harvest process including transportation and storage. Consequently, in order to prevent food borne diseases, some preservation way is required to stop or significantly slow down spoilage. This study evaluated the antifungal and antibacterial activities of some Indonesian plants against the food spoilage fungi and pathogen bacteria. The plants were extracted by distilled water and ethanol to investigate the antifungal and antibacterial activities by the broth micro-dilution methods. At 0.5 mg/ml concentration, *A. altilis* aqueous extract had the most significant antifungal activity against *Penicillium sp* with the antifungal activity (AFA) value of 140.36 ± 3.76%. In contrast, *C. burmanii* inhibited the growth of *Aspergillus nidulans* with the AFA value of 90.52 ± 15.97% in the same concentration. In the inhibition of *Escherichia coli* and *Salmonella thypii* growth, *A. altilis* ethanol extract gave the remarkable antibacterial activity with the MIC value of 0.025 mg/ml of each.

Keywords: Plant extracts, Antifungal activity, Antibacterial activity, Food preservatives

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Introduction

Microbial contamination has been leading to pathogenic infections and poor nutrition associated with weaning foods and the bacterial deterioration is become the one of most critical subjects in the production, processing, transport, and storing of food (He and Hwang, 2016). Fungi and bacteria are the contaminants cause of spoilage which commonly found in low-moisture food. Consumption of contaminated food with mycotoxins causes human severe and persistent mycotoxic which are apparent as cytotoxic, hepatotoxic, neurotoxic, teratogenic, carcinogenic, mutagenic and immunosuppressive effects (Diaz, 2005). Food preservation is the treating and handling of food to retain its nutrition value. The familiar ways to protect microbial spoilage of food commodities is the addition of synthetic or natural preservatives, which directly supplemented to the foods or incorporated in the food packages (Brul and Coote, 1999). Nowadays, there is a worldwide effort to minimize the use of chemical preservatives due to consumer preferences towards more natural and healthier products. Consequently, preference of consumers for foods without chemical preservatives has led to the discovery of new natural antimicrobial

and antioxidant preventing agents (Serra et al., 2008). It is widely well-known that some genus of the fungi such as *Penicillium*, *Aspergillus*, *Fusarium*, *Eurotium*, and *Alternaria* are the most producers of mycotoxins (Samson et al., 2004). *Aspergillus* has responsibility in the food and beverage industries due to their enzymatic activities. *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger* and *Aspergillus terreus* are some species which have considerable impacts on typically human infections among hundreds species in the *Aspergillus* genus. Species *Aspergillus nidulans* is often found and isolated from various types of food, either in fresh or processed food (Bertout et al., 2001). This species has been recognized to be harmful to humans and could grow hastily at 37 °C (Horre et al., 2002). They are commonly isolated from from cereals and cereal products (wheat, flour and bread, barley, rice, and sorghum), nuts, beans and spices (Pitt and Hocking, 1997). *Emericella nidulans* is listed as a producer sterigmatocystin, a mycotoxin more commonly associated with *Aspergillus versicolor* (Kawahara et al., 1994). *Penicillium* species are very common spoilage in foods and feeds that produces potential mycotoxin such as ochratoxin, citrinin, and paulin. This spoilage usually appears in the raw fruit, canned fruit, and cheese (Pitt, 2006). However some species of *Rhizopus* are probably not mycotoxins producers (Filtenborg et al., 1996). *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa* are the resistant strains bacteria which are being responsible for much morbidity and mortality worldwide (Lee et al., 2009). Natural preservatives such as essential oils, flavonoids, phenolic compounds, and microbial metabolites are the chemical agents derived from plants, animals, and microbes that could perform in food preservation against fungi and food borne bacteria (Prakash et al., 2014). They prevent the decomposition of products by inhibit microbial growth, oxidation and certain enzymatic reactions occurring in the foodstuffs (Singh et al., 2010). Some of them showed the significant antifungal activity against *Aspergillus* and *Rhizopus* genus (Malik et al., 2017).

The purpose of this study were to investigate the antifungal activity of some Indonesia plants extracts on the growth of *Penicillium sp*, *Aspergillus nidulans*, and *Rhizopus stolonifer* isolated from food, as well as their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

Material and Methods

Material

All plants were collected in Yogyakarta, Indonesia. Distilled water and ethanol were used as solvent for extraction. Assay media were prepared at Laboratory of Microbiology of Research Unit for Natural Products Technology, Indonesian Institute of Sciences, Yogyakarta, Indonesia. Potato dextrose agar (PDA) (Merck, Darmstadt, Germany) was prepared as the growth media.

Table 1. Indonesia medicinal plants and their part used

Plants	Part used
Artocarpus altilis (Parkinson) Fosberg	Leaves
Cinnamomum burmanii	Bark
Citrus hystrix DC.	Leaves
Cosmos caudatus Kunth.	Leaves
Cymbopogon citratus	Aerial
Foeniculum vulgare Mill.	Seed
Nigella sativa Linn.	Seed
Parkia Roxburghii G. Don	Seed
Physalis angulata L.	Fruit
Punica granatum L.	Peel
Stevia rebaudiana Cav.	Leaves
Zingiber officinale Roscoe	Rhizome

Sample preparation

The air-dried plant parts were exhaustively extracted with water and ethanol three times using ultra-sonication (ELMA Sonic, Germany) method in the room temperature. The supernatants were collected and concentrated under vacuum pressure using rotary evaporator (Buchi, Germany) to obtain aqueous and ethanol extracts. Aliquots of the extracts were diluted in sterile water for water extracts and DMSO (Merck, Germany) for ethanol extracts to get final concentration of 0.5 mg/mL.

Spoilage fungi isolation and identification

The contaminated fungi from traditional processed foods (sticky rice cake) were collected. A number of spoilage fungi were detected as *Penicillium sp*, *Aspergillus nidulans*, and *Rhizopus stolonifer*. Those fungi were then isolated and further used as the test organisms. The fungal contaminated food samples

was slashed and transferred with sterile forceps into Petri plates contained PDA (39 g/1000 mL distilled water) media. The plates were incubated at 27 ^{O}C for 4−7 days, depending on the type of fungi species. The isolated cultures were maintained on PDA media at 4 ^{O}C and stored for further assay (Samson et al., 2004). Cultures growing on PDA were determined according to macro and micro morphology as well as slide culture technique (Barnett and Hunter, 1998).

Media preparation for antifungal assay

The PDA media (39 g/1000 mL distilled water) which used as a medium for antifungal investigations was autoclaved at 121^{O}C for 15 min and then cooled to 45 ^{O}C. PDA was divided into equal volumes (30 mL), poured into Erlenmeyer (100 mL) flasks and each extract were added to the PDA to achieve the following concentrations: 0.5, 0.25, and 0.1 mg/mL. PDA with absence of extract was also prepared as negative control sample. Approximately 15 mL of PDA contained different extracts concentrations were poured in sterile Petri plate (Ø 9 cm).

Antifungal activity assay

Aqueous extract of plants was used for antifungal activity since these extracts would be applied as preservative to protect the spoilage fungi yielded in the sticky rice cake. The plates containing extracts concentrations, including control samples were inoculated in the core of the plate by spotting the 8 mm in diameter of fungal species until round inoculums were formed. Inoculation was performed in four replications with two inoculums per plate. After inoculation, Petri plates were closed properly and incubated at 27 ^{O}C. The evaluation of antifungal activity was carried out by measuring of the radial growth of the mycelium (in diameter) in each plate during 3 days for *Aspergillus nidulans* and *Rhizopus stolonifer* and 7 days for *Penicillium sp* in the presence of extracts. The antifungal activity (AFA) was calculated by the equation mentioned below:

$$AFA\ (\%) = (GC - GT) / GC \cdot 100$$

Where AFA is antifungal activity (%), GC is colony diameter on the control plate (mm) and GT is colony diameter on the test plate (mm) (Mori et al., 1997).

Antibacterial activity assay

The inhibition of bacterial strains growth was assessed through by micro-dilution method (Nisa et al., 2017). Bacterial strains of *Escherichia coli* FNCC 194, *Staphylococcus aureus* FNCC 0047, *Salmonella thypii* FNCC 015, and *Pseudomonas aeruginosa* were used for this assay. Each extract stock solutions was prepared at 10 mg/mL in DMSO and diluted onto 96-well plates containing microbial strains to obtain the series concentrations. The plates containing bacteria strains and samples were then incubated at 37 °C overnight. The commercially antibiotic, ampicillin (Merck, Germany) was used as a positive control. To evaluate the cell viability of bacteria in culture, approximately 50 µL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (Sigma Aldrich, USA) in isopropyl-HCl solution was subjected into each well and incubated further for 1 h.

Results and Discussion

Antifungal activity of aqueous extract from selected plants

The antifungal effect of several plant extracts was performed in order to investigate the capacity of those extracts as food preservative against some spoilage fungi which commonly contaminate Indonesian traditional sticky rice cake. The fungi which isolated from this food product are *Penicillium sp.*, *Aspergillus nidulans*, and *Rhizopus stolonifer*. The results of antifungal activity assay of the investigated plant extracts on *Penicillium sp.*, *Aspergillus nidulans*, and *Rhizopus stolonifer* spoilage fungi (Figure 1) are shown in Table 1. The morphology of mold on isolated fungi was observed under 40 X objective lens. The significant antifungal effect given by aqueous extracts were exhibited toward *Penicillium sp.* The strongest inhibitory activity was shown by *A. altilis* extract with the AFA value of 140.36 ± 3.76%. According to Mori et al (1997), the antifungal activity (AFA) value ≥ 75% is classified as very strong antifungal activity level. While, moderate antifungal activity level is notified by 75% ≥ AFA value ≥50%. Moderate antifungal activities against *Penicillium sp.* were demonstrated by *C. hystrix* and followed by *S. rebaudiana*, *N. sativa*, and *C. citratus* which ranged from 33.53 ± 31.67% to 44.57 ± 3.90% (Table 1). Sensitivity effect toward these selected plant extracts were exhibited by *Aspergillus nidulans* and *Rhizopus stolonifer* fungi. Almost all plant extracts had no significant antifungal activity against those *Aspergillus nidulans* and *Rhizopus stolonifer* fungi.

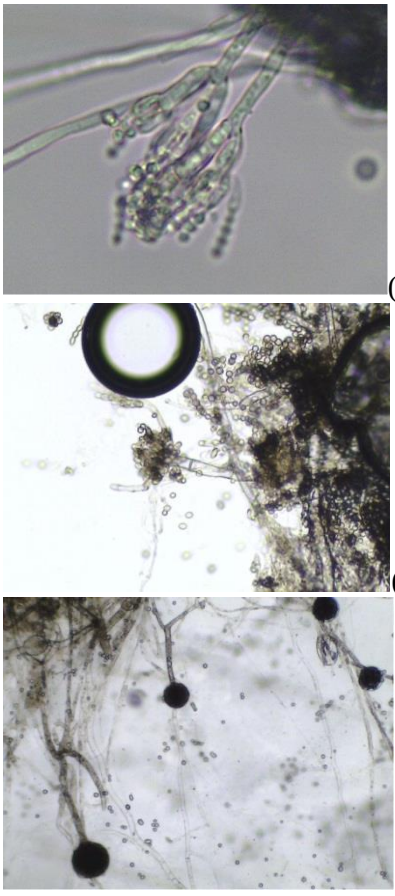

Figure 1 Microscopic image of (a) *Penicillium sp.*, (b) *Aspergillus nidulans,* and (c) *Rhizopus stolonifer* spoilage fungi (water 40 X)

Nevertheless, *C. burmanii* showed very strong antifungal activity against *Aspergillus nidulans* and moderate activity against *Rhizopus stolonifer* with the AFA value of 90.52 ± 15.97% and 61.75 ± 7.25%, respectively. In other hand, *A. altilis* demonstrated strong selected antifungal activity against *Rhizopus stolonifer*

The obtained results are reliable with the other reported studies which *A. altilis* extracts spot to have antifungal activity (Jayasinghe et al., 2004; Jagtap and Bapat, 2010). *A. altilis* extract exhibits antifungal activity due to its secondary metabolites composition. Mostly reported that steroids, terpenoids, flavonoids phytosterols, anthraquinone, and glycosides as the major phytochemicals of *A. altilis* are responsible on the growth inhibition of pathogen microorganism (Sivagnanasundaram and Karunanayake, 2015; Pradhan et al., 2012).

Table 2. Antifungal activity (AFA) of 0.5 mg/ml aqueous extracts against *Penicillium sp.*, *Aspergillus nidulans, Rhizopus stolonifer*

Indicator fungi	*Penicillium sp.*	*Aspergillus nidulans*	*Rhizopus stolonifer*
Plants	**AFA (%)**		
Artocarpus altilis (Parkinson) Fosberg	140.36 ± 3.76	2.20 ± 13.73	61.75 ± 7.25
Cinnamomum burmanii	NA	90.52 ± 15.97	40.76 ± 1.26
***Citrus hystrix* DC.**	33.53 ± 31.67	40.22 ± 11.08	NA
***Cosmos caudatus* Kunth.**	12.13 ± 2.50	NA	NA
Cymbopogon citratus	44.57 ± 3.90	NA	NA
***Foeniculum vulgare* Mill.**	22.35 ± 9.87	NA	NA
***Nigella sativa* Linn.**	33.84 ± 6.26	NA	NA
***Parkia roxburghii* G. Don**	12.44 ± 15.43	NA	NA
***Physalis angulata* L.**	17.40 ± 7.87	NA	40.43 ± 2.82
***Punica granatum* L.**	NA	NA	41.91 ± 0.80
***Stevia rebaudiana* Cav.**	43.29 ± 1.37	17.96 ± 6.16	NA
***Zingiber officinale* Roscoe**	16.73 ± 5.49	NA	NA

Data given are mean of three replicates ± Standard error.
NA: not active

One of the leading causes of illness and death in developed countries is uncontrolled food management effected by food borne pathogens (Mohanka and Priyanka, 2014). The major cause of food deterioration worldwide is produced by spoilage fungi (Jarvis et al., 1983). Several physical and chemical preservation treatments are continually developed to prevent spoilage in food. The results of the present investigation are an important step towards food protection strategies for antifungal activity against hazardous food borne species such as *Penicillium* and *Aspergillus*. Among the plants, *A. altilis* and *C. burmanii* would probably be an important candidate

plants for sources of food preservation. As the sensory role in food products, *C. burmanii* has acceptable preference in consumer. In other hand, this plant is also generally used as ingredients in many food products. Being natural and have therapeutic effect, medicinal plants should be not toxic for food consumption. Therefore, the additional research related to the safety of this plant application in food production is necessary to carry out.

Antibacterial activity of ethanol extracts from selected plants

Antibacterial effect of plant extracts toward food pathogen bacteria was also carried out. Bacterial contamination is related to the polluted raw materials, poor sanitation practices, lack equipment design, and deficient control of ingredients which causing food poisoning. Table 2 reported the MIC value of ethanol extracts from selected plants against several pathogen bacterial strains. Active antibacterial extracts were exposed as visually clear spots on the inhibition of the growth of test organisms in micro-plate wells. The average MIC values of the plants extracts ranged from 0.025 mg/mL to 0.2 mg/mL after 24 h of incubation. Among all the extracts, the *A. altilis* leaves extract exhibited the greatest antibacterial activity against pathogen bacterial strains tested. The good antibacterial activity of *A. altilis* was shown toward *E. coli* and *S. typhii* with the MIC value of 0.025 mg/ml of each and followed by *P. angulata* with the MIC value of 0.1 mg/mL of each. In contrast, the other plants showed insignificant antibacterial activity on the pathogen bacterial. In other hand, the antibacterial assay was also carried out toward plant aqueous extracts. However none showed the significant antibacterial activity (> 0.2 mg/ml).

Several biological activities of *A. altilis* and *P. angulata* had been reported by Wang et al (2007) and Soares et al (2006). *A. altilis* is known to be effective for the treatment of liver, hypertension and diabetes deseases. These pharmacological effects have been attributed to the components such as phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes (Wang et al., 2007). *P. angulata* contains some steroids such as physalins which are known to be beneficial in wound therapy (Soares et al., 2006).

The good antibacterial activity of *A. altilis* in the present result was confirmed and reported by Pradhan et al (2012) and Hesti et al (2016). However, the antifungal activity of those plants towards the spoilage fungi which responsibility to food borne illness is not much investigated.

Table 3. Antibacterial activity (MIC) of ethanol extracts against *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa*

Indicator bacteria	*E. coli*	*S. aureus*	*S. typhi*	*P. aeruginosa*
Plants	MIC (mg/ml)			
Artocarpus altilis (Parkinson) Fosberg	0.025	0.1	0.025	0.1
Cinnamomum burmanii	> 0.2	> 0.2	> 0.2	> 0.2
***Citrus hystrix* DC.**	> 0.2	> 0.2	> 0.2	> 0.2
***Cosmos caudatus* Kunth.**	> 0.2	> 0.2	> 0.2	0.2
Cymbopogon citratus	> 0.2	> 0.2	> 0.2	> 0.2
***Foeniculum vulgare* Mill.**	> 0.2	> 0.2	> 0.2	> 0.2
***Nigella sativa* Linn.**	> 0.2	> 0.2	> 0.2	> 0.2
***Parkia Roxburghii* G. Don**	> 0.2	> 0.2	> 0.2	> 0.2
***Physalis angulata* L.**	0.1	> 0.2	0.1	> 0.2
***Punica granatum* L.**	> 0.2	> 0.2	> 0.2	0.1
***Stevia rebaudiana* Cav.**	> 0.2	> 0.2	> 0.2	> 0.2
***Zingiber officinale* Roscoe**	> 0.2	> 0.2	> 0.2	> 0.2

Data given are mean of three replicates

Conclusion

The present investigation indicated that the extract of *A. altilis* exhibited much remarkable antifungal activity and antibacterial activity as well. *A. altilis* showed selective antifungal activity against the spoilage fungi, but had wide spectrum antibacterial activity on the microorganism tested. Other selective antimicrobial activities are also showed by *C. burmanii* and *P. angulata* In conclusion, the study showed that *A. altilis* and *C. burmanii* may be candidate plants for essential food preservative.

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Contribution of Authors

Nisa K: Conceived Idea, Data Collection, Data Analysis, Manuscript Writing.
Apriyana W: Data Collection, Data Interpretation
Rosyida VT: Designed Research Methodology, Data Analysis

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