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Research Article

The Inhibition of *Tobamovirus* by Using the Extract of Banana Flower

Penghambatan Tobamovirus *dengan Ekstrak Bunga Pisang*

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ABSTRACT

Some extract of the banana plants parts have been identified to produce a compound that has efficacy as traditional medicine and human virus inhibitor. There was no previous report on the use of the banana flower as the plants antiviral sources for plant pathogen. The objective of this study was to identify the potency of two types of the banana flower as the *Tobamovirus* inhibitor substance. The antiviral was prepared from the extract of Ambon banana (*Musa acuminata* Colla) flower and Klutuk banana (*Musa balbisiana* Colla) flower, as the comparison extract of *Mirabilis jalapa* L. leaf which is widely known to have antiviral on various plant viruses was used. This study applied the Complete Randomized Design with three replications. Collected data includes the virus incubation period and the inhibitor power upon the local necrotic symptom on indicator plant. Results of this study showed that the extract of Ambon and Klutuk banana flower was able to inhibit the *Tobamovirus* with inhibition levels of 86.34% and 91.22%.

Keywords: antiviral, Ambon banana, banana flower, Klutuk banana

INTISARI

Beberapa ekstrak bagian tanaman pisang diketahui memiliki kandungan senyawa yang berkhasiat sebagai obat tradisional dan zat yang dapat menghambat virus manusia. Belum pernah dilaporkan penggunaan bunga pisang sebagai sumber antiviral terhadap virus tumbuhan. Penelitian ini bertujuan untuk mengetahui potensi ekstrak bunga dua jenis pisang sebagai zat penghambat Tobamovirus*. Antiviral disiapkan dari ekstrak bunga pisang Ambon* (Musa acuminata *Colla*) *dan pisang Klutuk* (Musa balbisiana *Colla*)*, sebagai pembanding digunakan ekstrak daun* Mirabilis jalapa *L. yang sudah banyak diketahui mengandung antiviral pada berbagai virus tumbuhan. Penelitian ini dirancang menggunakan Rancangan Acak Lengkap dengan 3 ulangan. Data yang dikumpulkan meliputi masa inkubasi virus dan daya hambat gejala nekrotik lokal pada tanaman indikator. Hasil penelitian menunjukkan bahwa ekstrak bunga pisang jenis Ambon dan Klutuk mampu menghambat* Tobamovirus *dengan tingkat penghambatan sebesar 86,34% dan 91,22%.*

Kata kunci: antiviral, bunga pisang, pisang Ambon, pisang Klutuk

INTRODUCTION

Indonesia is one of the world's largest producers of bananas. In addition to its high nutrient content, various types of banana have been reported to contain compounds that are useful as anti-diabetes, anti-hypertension, antioxidant, antimicrobial, antiviral and anti-fungal, anti-diarrhoea, as well as anti-cancer (Jyothirmayi & Rao, 2015). Extract of *Musa acuminata* Colla flower can be used as antiviral for 1st type *Herpesvirus* and 2nd type *Herpesvirus* (Martins *et al*., 2009). Moreover, Swanson *et al.* (2010) reported that the isolated lectin, from banana fruit, can inhibit the replication of *Human Immunodeficiency Virus* (HIV-1).

Virus inhibitor compound is frequently found in plant extract, including in medicinal plant extract. Some compounds that isolated from the plant indicated the ability to inhibit the virus infectivity (Matthews, 1992). *Mirabilis jalapa* L. has been widely reported to have the potential as infection inhibitor for various types of plant viruses (Kubo *et al*., 1990). Various types of phytochemicals active in many types of plants such as flavonoid, terpenoids, lignin, tannin, alkaloid, coumarin, polysaccharides, protein, and peptides have been identified as various types of virus inhibitors (Jassim & Naji, 2003).

Along with the increasing problems of diseases caused by the virus on various important commodities,

the need for inexpensive and eco-friendly antiviral substance is also increasing. There has not been any report of antiviral substance for plant virus from the banana. The objective of this study was to identify the potency of two types of banana flower extract as the inhibitor substance for *Tobamovirus*. It was expected that the inhibitor substance obtained in this study could be further developed as a component in the plant virus diseases management. Some of the reported data have been communicated orally in the National Seminar and XXIV Congress of the Indonesian Phytopathological Society (PFI) not necessary (Nurviani *et al*., 2017).

MATERIALS AND METHODS

Source of Inoculum

Tobamovirus inoculum was prepared on Boyolali Tobacco leaves with mosaic symptom from Kedu, Temanggung that isolated on *Chenopodium amaranticolor* H.J. Coste & A. Reyn plant and multiplied on the tobacco plant. The nucleic acid trace of the *Tobamovirus* that already had been confirmed through sequencing DNA by Endarsih *et al*. (2017). The single lesion resulted from the isolation was inoculated mechanically to the 5-weeks old tobacco and maintained in the laboratory.

Preparation of Banana Flower Sample

The antiviral material used was banana flowers on Ambon (AAA) and Klutuk (ABB) bract which was dark red colour, and *M. jalapa* leaves as the comparison. The banana flower was originated from Gamping, Sleman, Yogyakarta which is located at 100–499 a.s.l. After sprayed using alcohol 70 % and wiped using tissues, the fresh banana flower was chopped into little pieces for extraction.

Extraction of Antiviral Compound

As much as 50 g of the banana flower wrapped in filter paper, then was put into the soxhlet an instrument with 150 ml ethanol solvent. The extraction process was carried out in four circulations, and then the extract was concentrated by using rotary evaporator at 40°C until dry. The aquadest was added to reach 10 ml volume (Rini, 2004). The extract was stored at -20ºC as to become ready to use for the antiviral testing (Martins *et al*., 2009). The extract turbidity level was measured at the maximum red colour wavelength (750 nm) with a spectrometer.

The *M. jalapa* extract was made based on the method Kubo *et al.* (1990). As much as 1 g of *M. jalapa* leaves was cleansed in running water and then crushed in the mortar and added with 10 mL of 0.01 M pH 7 cold phosphate buffer. Then filtered by using sterile cotton and it was ready used as the comparison in the antiviral testing. This extract of *M. jalapa* is a dilution of 10. Based on the Martono's research (2000), that the best *M. jalapa* extract is on the leaves with dilution of 10.

Phytotoxicity Testing of the Antiviral Compound

The testing phytotoxicity was carried out by rubbing the extract of Ambon and Klutuk banana flower onto the leaf of *C. amaranticolor* plant. Each treatment was applied to 3 specimen leaves. The symptom observation was carried out within 14 days in order to identify the influence of the phytotoxicity of banana flower extract to specimen plant.

The Antiviral Effectiveness Testing to the Tobamovirus

The indicator plant was previously put in the darkroom in 24 hours before inoculation, the darkness level was measured by using the food candle up to the needle reaching the number 0. Virus sap prepared with 1 gram of *Tobamovirus* leaf isolate crushed with 10 ml 0.01 M pH 7 cold phosphate buffer and filtered with sterile cotton. Then, the virus isolate was diluted to 10^{-4} with aquadest. Next, the virus sap was added with the antiviral (ratio 1:1 ml) on each extract treatment. Inoculation on the specimen plant was done by rubbing virus sap with hand gloves. As the control, the plant was inoculated by using the virus without antiviral treatment. Afterwards, the indicator plant leaves were washed off with aquadest. Inoculation was carried out to the 3 selected indicator plant leaves (as the replication). The virus incubation was observed and the number of lesions and the leaves area were calculated. Data was obtained by calculating the number of lesions up to 7 days of observations. The inhibition power was calculated with the formulation of Lu *et al*. (2013):

$$\textit{Inhibition Power (\%)} = [(K-P)/K] \times 100\%$$

K is the average local lesions on the indicator plant without extract treatment (control), and P is the average local lesions with extract treatment. Obtained data were analyzed statistically by using the *Duncan's Multiple Range Test* (DMRT) with the significant difference at 0.05 to identify the influence of each treatment.

RESULTS AND DISCUSION

Banana Flower as Source of Antiviral Substance

Ambon banana flower is dark red, while Klutuk banana flower has a bright red colour. The banana flower used a female flower, which was taken when the banana fruits were already formed (Figure 1). The bract extracted was only those in the red part.

Extract Banana Flower

The absorbance value of the Ambon banana flower extract was 0.828 nm and the Klutuk banana flower was 0.101 nm.

Phytotoxicity Testing on the Antiviral Compound against Tobamovirus

The phytotoxicity testing result showed that there was no damage to the leaf rubbed with banana flower extract treatment (Figure 2). This indication showed that the extract used as the antiviral material was not toxic to the specimen plant. Therefore, the extract of the banana flower was potential as an antiviral.

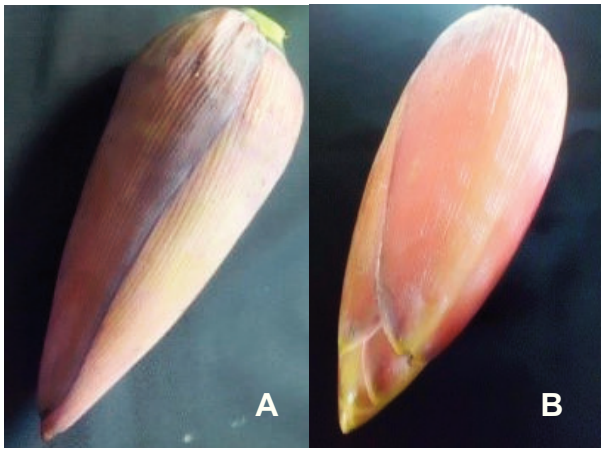

Figure 1. The banana flower as sources of antiviral from Sleman (A) Ambon banana, (B) Klutuk banana

The Antiviral Effectiveness to the Tobamovirus

The incubation period was within 5 days treatments of the extract of Ambon and Klutuk banana flowers. Such period was the same as in control plant. Meanwhile, in the extract of *M. jalapa*, the incubation period could be extended up to 7 days after inoculation (HSI). This is because virus require different amounts of time to move from cell-to-cell in the leaf via the plasmodesmata (Matthews, 1970).

Test results (Table 1) showed that all treatments of extract had no effect on lesion average. However, each treatment of extract can reduce the formation of local lesions on the leaves. The inhibiting power of Klutuk banana flower extract was 91.22% and Ambon was 86.34%. Similarly, the inhibitory power of each extract treatment was significantly different from the one without extract (Figure 3).

Table 1. The inhibition power extract banana flower to *Tobamovirus*

Extract Treatment	Lesion Average*) **)	Inhibition (%)**)
Control	1.76a	0.00c
Mirabilis jalapa	0.07b	95.74a
Ambon Banana Flower	0.24b	86.34b
Klutuk Banana Flower	0.15b	91.22ab

*) the average of 3 replications per leaf area
**) a number followed by the same letter in the same column shows no real difference in the 0.05 DMRT test

Figure 2. The result of phytotoxicity test of banana flower extract on *Chenopodium amaranticolor*: (A) Ambon banana flower, (B) Klutuk banana flower

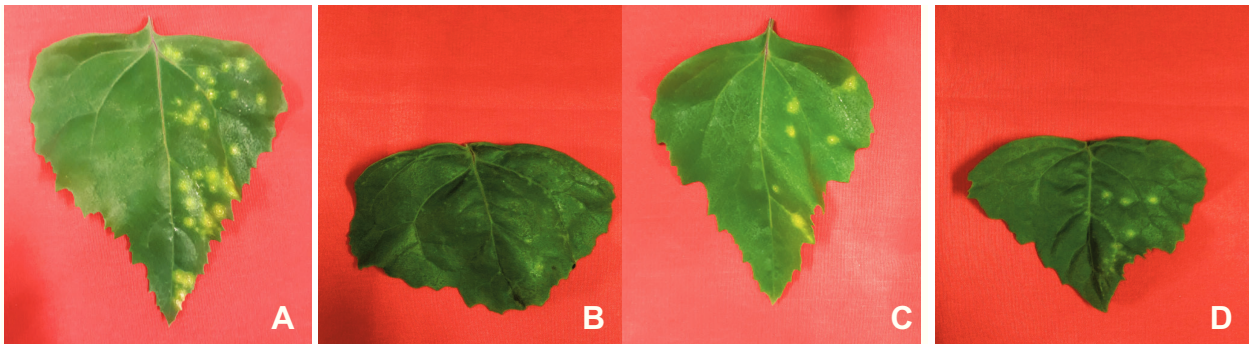

Figure 3. The reaction of *Tobamovirus* with antiviral compounds from (A) H_20, (B) *Mirabilis jalapa*, (C) Ambon banana flower, (D) Klutuk banana flower on *Chenopodium amaranticolor* leaves showed by local lesions after incubated on 3 day after infestation

The possibility of the phenolic compound contained in the banana flower such as tannin could play the role of antiviral. According to Cheo and Lindner (1970), that tannic acid can inactivate to *Tobacco mosaic virus* (TMV) RNA as well as intact TMV in vitro. The inactivation of whole TMV appeared due to a reaction of the tannic acid with the RNA core. Matthews (1970) also reported that the phenolic compound found in some plants can paralyze protein. The phenolic compounds are widespread in plants and frequently reach in high concentrations. Phenol can directly react to certain groups in the protein with a hydrogen bond. Oxidized phenol cause the loose of some virus infectivities (Hampton & Fulton, 1961; Matthews, 1970).

To our knowledge, this article is the first report of the existence of antiviral in banana flower which is effectively inhibited the plant virus. Therefore, further research in the identification of an antiviral compound, the identification mechanism, and effectiveness to another type of plant virus, as well as the proper formulation in the virus management is required.

CONCLUSION

The extract of Ambon and Klutuk banana flowers inhibited *Tobamovirus* at the level of 86.34% and 91.22%.

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