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Quassinoids isolated from *Brucea javanica* inhibit pepper mottle virus in pepper

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ABSTRACT

A green fluorescent protein (GFP)-tagged pepper mottle virus (PepMoV) based leaf-disc method and systemic host method were developed to identify antiviral agents. Preliminary experiments using a PepMoV-GFP based leaf-disc method led to the isolation of five quassinoids, including brusatol (**1**), bruceantin (**2**), brucein A (**3**), bruceantinol (**4**), and brucein B (**5**), from the CH_3OH extract of *Brucea javanica*. All isolated compounds exhibited inactivation effects in systemic host plants, and compounds **3** and **4** were potent, with a minimum inhibitory concentration of 10 μM. Furthermore, compound **3** was found to have a protective effect at the tested concentration of 40 μM.

1. Introduction

Peppers (*Capsicum* spp.), one of the most widely used ingredients in cuisines around the world, are afflicted with diverse diseases which can result in reduced fruit quality or yield. Pepper diseases are caused by a variety of pathogenic microorganisms such as bacteria, fungi, and viruses. In particular, diseases caused by plant viruses are a main constraint limiting the sustainable production of pepper (Pernezny et al., 2003). A number of viruses, including cucumber mosaic virus (CMV), pepper mottle virus (PepMoV), and tobacco mosaic virus (TMV), have been reported to infect peppers (Choi et al., 2005; Green and Kim, 1991). Specifically, PepMoV, belonging to the genus *Potyvirus* in the family *Potyviridae* and consisting of a filamentous particle with a positive single-stranded RNA genome, predominantly infects *Capsicum* species (Han et al., 2006; Kim et al., 2009; Shukla et al., 1994).

Most viruses are transmitted by insect vectors, replicate within host cells, move from cell to cell, and can damage host plants (Carrington et al., 1996; Hogenhout et al., 2008). Symptoms of viral infection include mosaic, mottled, and crinkled leaves and malformed fruits. For the control of viral diseases, using insecticides, culling of infected hosts, and using resistant cultivars are known to be the only methods for preventing viral infection because there are rarely antiviral agents that directly interrupt the infection process (Pernezny et al., 2003). Thus, there is an urgent need to develop effective antiviral agents.

In the last decade, a few effective antiviral agents against TMV and CMV have been reported (Chen et al., 2009a; Ge et al., 2012; Han et al., 2015; Hu et al., 2012; Yan et al., 2010; Zhao et al., 2013). The half-leaf method, comparing number of local lesions of treated and non-treated regions on an infected plant leaf (Samuel, 1931) is commonly used to discover antiviral agents against viruses causing local lesions on hosts, including TMV and CMV which infect *Nicotiana glutinosa* L. and *Chenopodium amaranticolor* Costs & Reyn, respectively. In contrast, pepper (*Capsicum annuum* L.) infected by PepMoV exhibits systemic symptoms without local lesions, so

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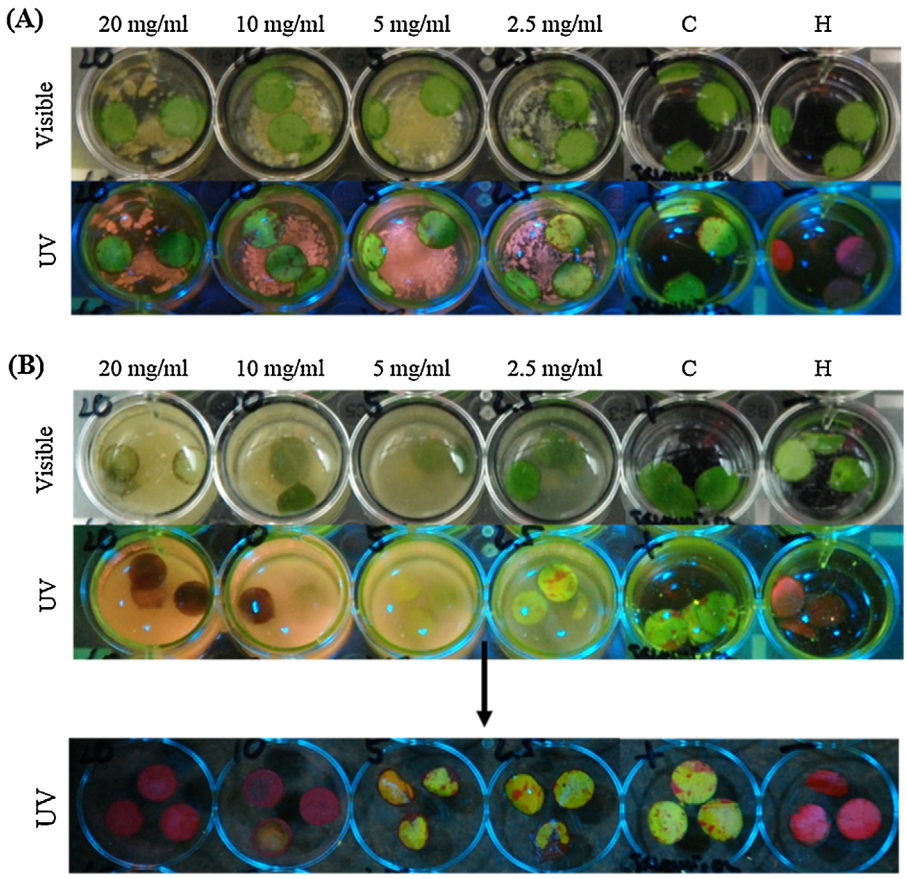

Fig. 1. Photograph of leaf-discs treated with the CH_3OH extract of *B. javanica* at different concentrations (20, 10, 5, and 2.5 mg/mL). The photographs were taken at (A) 0 h and (B) 7 days under UV and visible lights. The bold arrow indicates the tested leaf-discs at 7 days without culture medium under UV light. C, control (leaf-discs with DMSO); H, healthy.

antiviral effects against PepMoV infecting pepper cannot be evaluated using this assay. Therefore, the half-leaf method is only applicable to a limited number of viruses that produce local lesions on hosts.

The green fluorescent protein (GFP) tagged PepMoV, SP6PepMoV-Vb1/GFP (PepMoV-GFP), is a modified virus in which the gene encoding GFP is inserted into the coding region and can systemically infect and express GFP in whole plants (Lee et al., 2011). By using PepMoV-GFP, the existence of virus can be easily detected under UV light at an earlier time point than is possible using visual virus-induced symptoms in host plants infected by PepMoV-GFP. Therefore, antiviral effects can be simply evaluated in host plants systemically infected by PepMoV-GFP. Furthermore, a PepMoV-GFP based leaf-disc method can, rapidly and with high capacity, identify antiviral agents and requires only small sample amounts.

In the present study, 500 plant and fungal extracts were tested for their anti-PepMoV potentials using a PepMoV-GFP based leaf-disc method. The CH_3OH extract of *Brucea javanica* (L.) Merr was found to inhibit viral activity. *B. javanica*, belonging to the family Simaroubaceae, is distributed throughout East Asia, and its fruits have been used in traditional herbal medicine as antipyretic and detoxifying agents (Chen et al., 2013). It was previously reported that the CH_3OH extract of *B. javanica* exhibited anti-TMV activity, and a series of quassinoids, isolated from *B. javanica*, showed significant inhibition against TMV and potato virus Y (PVY) (Chen et al., 2009b; Shen et al., 2008; Yan et al., 2010). Therefore, we systematically investigated the quassinoids from *B. javanica* in an attempt to identify effective antiviral agents against PepMoV. Herein, we describe the antiviral activity of the extract and five quassinoids (**1**–**5**) isolated from *B. javanica*, and report the first instance of evaluation of their antiviral activities against PepMoV in host plants systemically infected by PepMoV-GFP.

2. Materials and methods

2.1. General experimental procedures

Column chromatography was performed using silica gel (230–400 mesh, Merck, Darmstadt, Germany), C_{18} reversed-phase (RP) silica gel (12 μm, YMC, Kyoto, Japan), Sephadex LH-20 (18–111 μm, GE Healthcare AB, Stockholm, Sweden), and Diaion HP-20 resin (Supelco, Bellefonte, PA). Thin-layer chromatography was performed using precoated silica gel 60 F254 plates (0.25 mm, Merck). For the extraction and isolation of samples, all organic solvents used were extra-pure grade (Dae-jung, Siheung, Korea). NMR spectra were obtained using a Varian 500 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ values. ESIMS was performed on a Waters Q-TOF micromass spectrometer.

2.2. Preparation of screening materials

Hot pepper (*Capsicum annuum* L., P915 inbred line, Nongwoo Bio Co., Ltd., Suwon, Korea) was grown in a greenhouse of Seoul Women's University, Seoul, Korea, and GFP tagged PepMoV (pSP6PepMoV-Vb1/GFP) was obtained from the Plant Virus GenBank, Seoul Women's University, Seoul, Korea.