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Note

Antifungal Activity of Plumbagin Purified from Leaves of *Nepenthes ventricosa* x *maxima* against Phytopathogenic Fungi

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A kind of naphthoquinone, plumbagin was purified and identified from the leaves of *Nepenthes ventricosa* x *maxima* through solvent extraction, silica gel column chromatography, and recrystallization. The yield (0.51 %) was higher than that of the root of *Plumbago scandens* (0.26%), *P. capensis* (0.15%), and *N. thorelii* (0.092%). It exhibited antifungal activity against all plant pathogenic fungi tested, *Alternaria alternata*, *Aspergillus niger*, *Bipolaris oryzae*, *Fusarium oxysporum*, *Phytophthora capsici*, *Rhizoctonia solani*, *Rhizopus stolonifer* var. *stolonifer* and *Sclerotinia sclerotiorum*. The minimum inhibitory concentration values ranged from about 4.8 to 56.6 μg/ml against the above eight fungi and *R. solani* was the most sensitive.

Keywords **:** *Nepenthes ventricosa* × *maxima*, antifungal activity, plumbagin

Phytochemicals may be an alternative to currently used fungicides to control plant pathogenic fungi, because they constitute less environmental risk and mammalian toxicity (Cho et al., 2006; Hwang et al., 2005; Lee and Lee, 2005; Lee et al., 2005). Quinones occurring in higher plants have a good to moderate antifungal activity against phytopathogenic fungi (Lee and Lee, 2005; Meazza et al., 2003). Phenols and guaiacols extracted from wood vinegar of *Cryptomeria japonica* have a strong antifungal activity against four plant pathogenic fungi (Hwang et al., 2005).

Nepenthes are carnivorous plants using their specialized leaves as passive pitfalls and trap arthropods (Juniper et al., 1989). Phytochemically, naphthoquinones are characteristic of *Nepenthes* and are chemotaxonomic markers within the *Nepenthelas*. Several naphthoquinones have been isolated and identified from the roots of *N. rafflesiana* (Cannon et al., 1980), *N. thorelii* (Likhitwitayawuid et al., 1998), *N. gracilis* (Aung et al., 2002), and *N. insignis* (Rischer et al., 2002). A kind of naphthoquinone, plumbagin has insect antifeedant (Kubo et al., 1980), cardiotonic (Itoigawa et al., 1991), anticancer (Parimara and Sachdanandam, 1993), antimicrobial (Didry et al., 1994), and antimalaria activities (Likhitwitayawuid et al., 1998). However, no detail study has described yet on the antifungal activity of plumbagin against plant pathogenic fungi. The present paper reports on the inhibition of eight plant pathogenic fungi by plumbagin isolated from the leaves of *N. ventricosa* × *maxima* hybrid.

HPLC was performed with Shimadzu LC 10 AD pump, SPD 10 A UV-VIS detector, and the column used was Shim-pack CLC-ODS (M) (4.6 × 250 mm) with a detection wavelength of 254 nm. The mobile phase was methanol and water (80:20, v/v) with 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min. EIMS was measured using a HP 5890 GC/MS spectrometer using HP5-MS column. Injector temperature was 270°C, and column temperature was 70°C for 1 min at the beginning and then raised to 120°C at rate 12°C/min and hold 1 min, 200°C at rate 15°C/min and hold 1 min, and 300°C at rate 20°C/min and hold 15 min. ^{1}H NMR spectroscopy was performed using a Bruker AC-300 at 300 MHz using $CDCl_3$ as solvent.

The lyophilized leaves of plant (100 g) was ground and extracted with methanol overnight at room temperature. The methanol extract was re-extracted with hexane. The extract was subjected to flash chromatography on silica gel using a hexane and ethyl acetate (2:1, v/v) solvent system and detection of antifungal compound was achieved by bioassay. After collection of active fractions, further purification was performed by recrystallization with hexane, yielding 0.51 g of orange crystalline solid.

The plant pathogenic fungi were acquired from the Korean Agricultural Culture Collection (KACC). The antifungal activity of the active compound which was resolve in methanol was tested by the modified method of Daouk et al. (1995) using potato dextrose broth. The minimum inhibitory concentration (MIC) was the lowest concentration of plumbagin that completely inhibited growth of the fungi and the antifungal testing was performed in triplicate.

The presence of plumbagin in methanol extract of the leaves of *N. ventricosa* × *maxima* was estimated by HPLC with a retention time of 6.75 min. The peak area was calculated by comparing with an authentic sample of

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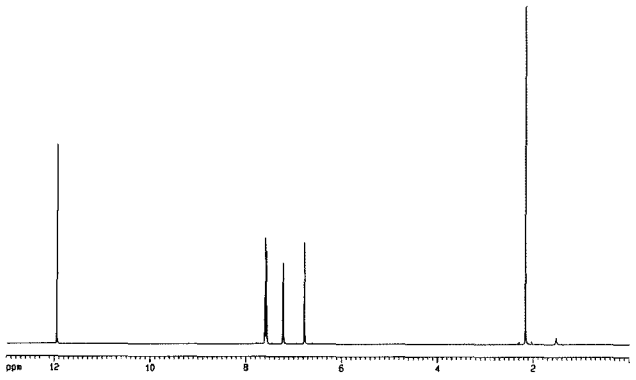

Fig. 1. ^{1}H NMR spectrum of plumbagin purified from *N. ventricosa* × *maxima*.

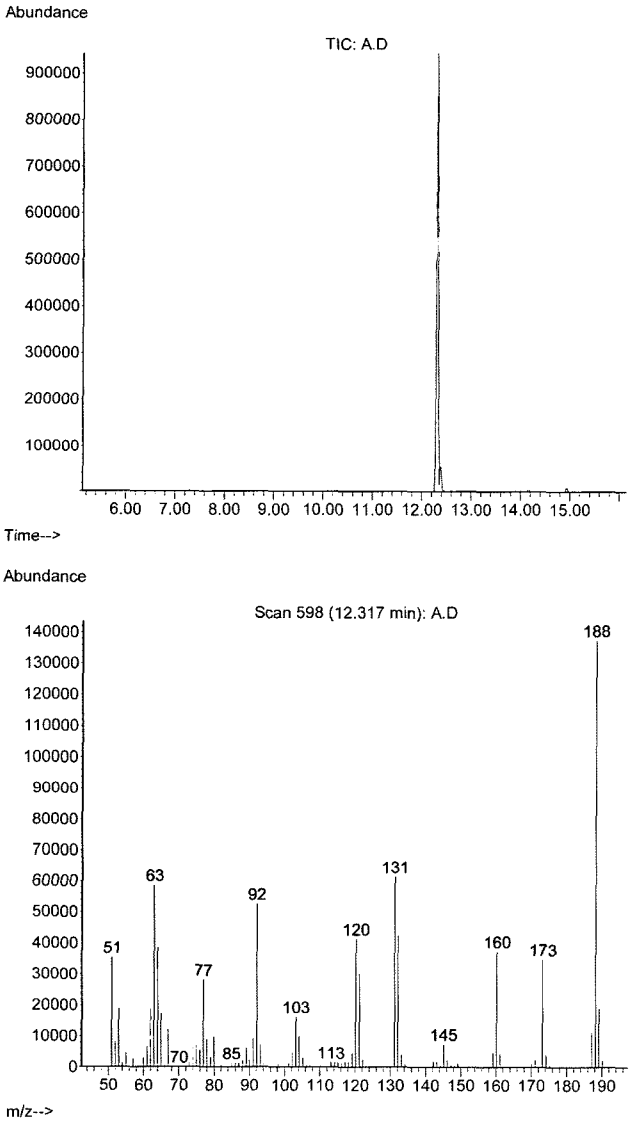

Fig. 2. EI-MS spectrum of plumbagin purified from the leaves of *N. ventricosa* × *maxima*.

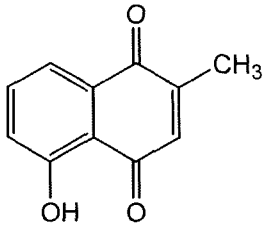

Fig. 3. Structure of plumbagin isolated from *N. ventricosa* × *maxima*.

plumbagin purchased from Sigma-Aldrich (Product No. P7262). Identification of plumbagin was carried out by ^{1}H NMR (Fig. 1) and GC/MS spectrum with a retention time of 12.32 min (Fig. 2). ^{1}H NMR ($CDCl_3$) δ (ppm); 2.18 (s, 3H), 6.79 (m, 1H), 7.25 (m, 1H), 7.60 (dd, 2H), and 11.96 (s, 1H). EI-MS *m/z* (intensity, %); 188 (100), 173 (26), 160 (27), 131 (44), 120 (30), 92 (39), and 63 (43). These spectral data were identical to those of a reference sample (P7262) purchased from Sigma-Aldrich (St. Louis, USA).

The plumbagin yield (0.51%) on dry weight basis was higher than that of the root of *Plumbago capensis* (0.15%) (Itoigawa et al., 1991), *P. scandens* (0.26%) (Paiva et al., 2003), and *N. thorelii* (0.092%) (Likhitwitayawuid et al., 1998). The reported plumbagin yields in cell cultures of *Drosophyllum capensis* and *D. natalensis* were 0.0004% (Crouch et al., 1990) and in callus cultures of *P. zeylanica* (Heble et al., 1974) and *P. rosea* (Komaraiah et al., 2003) on fresh weight basis were 0.0001-0.003% and 0.02-0.035%, respectively. It indicates that the use of this high-yielding plant for the initiation of tissue culture may be the way to succeed in an increasing of secondary metabolite production by plant tissue cultures.

The antifungal activity and the antifungal spectra of plumbagin were determined with eight plant pathogenic fungi. The plumbagin could inhibit all examined plant pathogenic fungi including Oomycota (*P. capsici*), Zygomycota (*R. stolonifer* var. *stolonifer*) and Deuteromycota (*A. alternata*, *A. niger*, *B. oryzae*, *F. oxysporum*, *R. solani*,

Table 1. Minimal inhibition concentration (MIC) of plumbagin purified from the leaves of *N. ventricosa* × *maxima*

Fungus	KACC No.	MIC (μg/ml)
Alternaria alternata	40019	27.0 ± 5.2
Aspergillus niger	41858	13.5 ± 0.3
Bipolaris oryzae	41025	9.7 ± 0.4
Fusarium oxysporum	40053	21.1 ± 5.0
Phytophthora capsici	40157	56.6 ± 5.3
Rhizoctonia solani	40136	4.8 ± 0.1
Rhizopus stolonifer var. *stolonifer*	41364	16.6 ± 2.8
Sclerotinia sclerotiorum	40457	12.1 ± 0.2

S. sclerotiorum), of which *R. solani* was the most sensitive. Previously, it had been reported that plumbagin was only weakly active against plant pathogenic fungi such as *S. libertiana* (= *S. sclerotiorum*) and MIC values described roughly, as 100 μg/ml (Kubo et al., 1980). However, in this experiment, plumbagin exhibited potent antifungal activity except *P. capsici* and more precise MIC values were listed in Table 1. These results indicate that the plumbagin might be promising antifungal agents effective on the biocontrol of plant diseases with reduced environmental contamination and residual toxicity.

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