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Article

Antifungal and Antioxidative Activities of *Yucca smallina* Fern.

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The antifungal activity of crude methanolic extract and fractions from *Yucca smalliana* Fern. leaves, roots and flowers were investigated *in vitro* against a panel of plant pathogenic fungi. The minimal inhibitory concentration (MIC) was determined by an agar dilution method. Preliminary liquid culture and agar plate assays showed that the growth of *Fusarium oxysporum*, *Phytophthora capsici*, *Rhizoctonia solani* and *Botrytis cinerea* were inhibited by *Y. smalliana* extracts. The extracts from flowers and leaves showed antifungal activity of 64.0% and 34.0% against *F. oxysporum*, 66.0% and 62.0% against *P. capsici*, and 27.0% and 41.0% against *B. cinerea*, respectively. The methanolic extract from *Y. smallina* leaves in distilled water was fractionated using solvents of increasing polarity: hexane, ethyl acetate and butanol. These fractions had a broad spectrum of antifungal activity, found to reside entirely in the butanol and aqueous fraction. The aqueous fraction showed inhibition rate of 60.0, 67.8, 84.6 and 58.3% against *F. oxysporum*, *R. solani*, *C. gloeosporioides*, and *B. cinerea*, respectively, and the butanol fraction showed 36.0, 46.0, 66.1 and 58.3%, respectively. Phenolics (e.g. flavonoids, steroids and terpenoids) were observed in the thin layer profile of the different fractions. Leave extract showed a prominent antioxidant activity totally scavenging the free radical of DPPH at a concentration of 1 mg/ml.

Key words: *Yucca smalliana, antifungal activity, antioxidant, phenolics, methanol extract*

The continued frequent or excessive use of organic synthetic chemicals to control different pests exerted serious environmental problems. Those problems can be in part solved by using agricultural and biological control approaches e.g. breeding for disease-resistant varieties, and natural pesticides. The biotic pesticides are natural products that protect crops from blight, vermin, weeds, host-resistant microbes, natural plants, natural enemies, and other biological control agents from the natural environment. Biopesticides are slow in their control effects compared with synthetic chemicals and should have different control agents developed against different pests. Despite these disadvantages, they can target specific pests without causing ecological damage. In addition, they are not toxic to humans and could be used even during the harvesting season.

Yucca smalliana Fern. is an evergreen perennial that belongs to the family Agavaceae. The fibers collected from the leaves are used as textile materials and in different handcrafts. Native to southern and northern America, this plant is also raised for medicinal or decorative purposes. Plants in this genus are notably resistant to honey fungus. *Yucca* is commercially used as a saponin source. It is also a rich source of polyphenolics,[1)] including resveratrol and a number of other stilbenes (yuccaols A, B, C, D and E). These stilbenes play important roles in plants, especially in heartwood protection as part of both constitutive and inducible defense mechanisms, and in dormancy and growth inhibition. Certain stilbenoids, besides being toxic to insects and other organisms, have mammalian antifeedant and nematicidal properties.[2)] *Yucca* phenolics are also anti-oxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species that stimulate inflammatory responses.[1)]

Cucumber damping-off is an important soil-borne disease caused by infection with *R. solani* roots rot. The fungus infects the plant when grown on slightly acidic soil. Its incidence during the seedling stage is serious, and could cause complete crop losses.

Phytophthora blight of red pepper is caused by *P. capsici.* This fungus infects the whole plant resulting in a 20-30% decrease in the annual harvest. Powdery mildew is the most commonly known plant fungal infection. Powdery mildew is characterized by a white powdery growth on the foliage. Many fungi are responsible for this disease. It is common in poor ventilated areas that show great day and night temperature differences between particularly.

In this study methanolic extracts from different parts of *Y. smallaina* (leaves, roots, flowers) were investigated for their potential antifungal activity against plant pathogens that cause valuable economic losses. The plant extracts were assessed against *R. solani* roots rot, Phytophthora blight and Powdery

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mildew. The antioxidant activity of the different extracts and fractions was also monitored.

Materials and Methods

Plant material collection, preparation and extraction. Plant material was collected from the farm of Chonnam National University in 2001. It was divided in parts (leaves, roots, and flowers), air dried and course ground (Table1). Leaves were collected at different periods during different growth intervals to measure their seasonal variation of the biological activities. Each part was extracted twice at room temperature using 80.0% methanol (Table 1). Methanol was removed under reduced pressure in a rotary evaporator. The remaining aqueous extract was lyophilized to obtain the final sample, which was subjected to different biological assaying.

Organisms and media. Culture media used for the detection of antifungal activity were PDB (Potato Dextrose Broth, Difco, USA), PDA (Potato Dextrose Agar, Merck, Germany), and BHI (Brain Heart Infusion, Difco, USA). The microorganisms used for the detection of antimicrobial activity were plant pathogenic fungi, *Didymella bryoniae* (KACC 40578), *Fusarium graminearum* (KACC 40532), *Fusarium oxysporum* (KACC 40053), *Rhizoctonia solani* (KACC 40101), *Botrytis cinerea* (KACC 40574), and *Phytophthora capsici* (KACC 40483). *R. solani* used for the antimicrobial test was obtained from Gyeongsang National University. *P. capsici* culture medium was composed of 20.0% V-8 agar (20.0% juice, 0.03% $CaCO_3$, 2.0% agar) at a controlled pH (6.0), and PDA culture medium was used for other plant pathogenic fungi.

Table 1. Weights of methanolic extract obtained from different parts of *Y. smalliana*

Plant part (harvest date)	Fresh plant material (g)	Methanolic extract (g)
Leaves (Jun. 26, 2001)	100	5.8
Leaves (Aug. 26, 2001)	100	8.2
Leaves (Nov. 02, 2001)	1800	200
Leaves (Jan. 02, 2002)	102	11.0
Leaves (Mar. 02, 2002)	103	15.5
Leaves (May. 02, 2002)	100	12.4
Flowers (Jun. 26, 2001)	1068	54.7
Roots (Jun. 26, 2001)	820	114.1

Minimum inhibition concentration (MIC) agar dilution test. Culture media were prepared in two ways: sterilizing PDA culture media containing different concentrations of *Y. smalliana* extracts (0, 0.0125, 0.025, 0.05, 0.1, and 0.25%); adding 0, 0.0125, 0.025, 0.05, 0.1, and 0.25% of *Y. smalliana* extracts to the sterilized PDA culture media. Inoculated filter paper discs (6 mm diameter; Oxoid) were placed on the agar surfaces. The plates were observed after 48 h at 30°C. The

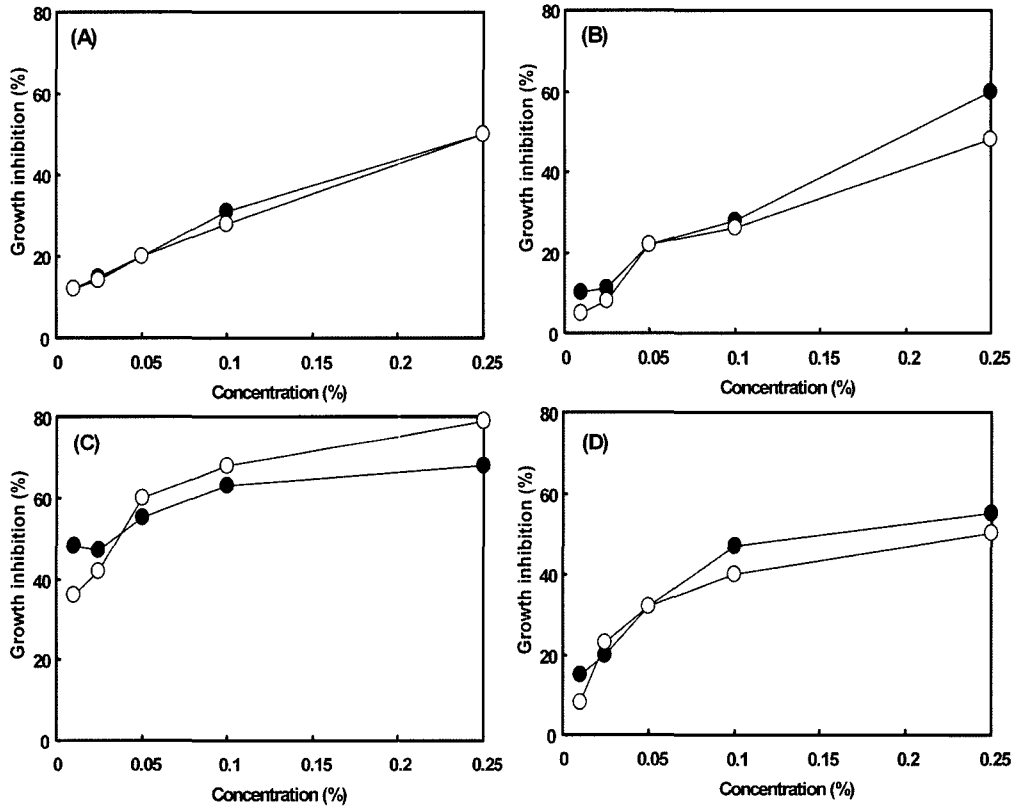

Fig. 1. Effect of methanolic extract of *Yucca* leaves on the growth of plant pathogens such as *F. oxysporum* (A), *P. capcisi* (B), *R. solani* (C), and *B. cinerea* (D). ○, Sample added before PDA sterilization; Sample added after PDA sterilization.

growth rates of the control Petri dishes and the experimental Petri dishes were compared, and then inhibition rates were calculated. All tests were performed in triplicate.

Antifungal activity of the extracts against cucumber damping-off. Cucumber seeds used for the test were winter-bearing *Cucumis sativus* of Heungnong Yuksung. 50 m*l* of PDB were poured into 250 m*l* Erlenmeyer flask, sterilized and inoculated with PDA culture of *R. solani*. The inoculum was cultured at 28°C for 5 days. *R. solani* hyphae produced on the surface of the culture solution were homogenized in sterile water for about 2 minutes and 25 mg/kg of it was inoculated in soil.[3]

Cucumber seeds, which were sterilized in 2% NaClO for 10 minutes, were sowed in sterile bed soil [121 for one hour 3 times, (4 seeds per pot)]. Five days later, it was infected with *R. solani* (25 mg/kg). 15 m*l* of 1.0% *Y. smallina* extracts were then added to measure their antifungal activities during the growth of the infected cucumber seedlings.

Antifungal activity of extracts against red-pepper *Phytophthora* blight. *P. capsici* was inoculated in the prepared culture medium and allowed to grow at 26, to cover the rim of the plate. Using a glass rod, aerial hyphae were removed and cultured away from fluorescent lamp for 3 days to allow formation of sporangium. 10 m*l* of sterile water was added. The mixture was chilled for an hour to discharge planospore from the sporangium to be able to recover them.[4] Fifty days old red-peppers were inoculated with *P. capsici* planospores. Each inoculated pot was treated with 20 m*l* of yucca extracts (1.0%) to measure their biological control against this fungus.

Antioxidant Activity. Following an established methodology,[5,6] the antioxidant activity of the different extracts was expressed as scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Sample solution (0.1 m*l*) and 0.9 m*l* of 0.15 mM solution of (DPPH) in ethanol were mixed in a test tube and allowed to react at room temperature 30 min. Absorbance at 517 nm was measured, and the percent of activity was calculated.

Fractionation of *Y. smalliana* leaves methanolic extracts. The methanolic extract of *Y. smalliana* leaves (fresh weight 200 g collected on November 2nd, 2001) was dissolved in distilled water and fractionated with the aid of a separating funnel using hexane, EtOAc and BuOH. Each fraction was subjected to biological assaying after removal of solvents.

Thin layer chromatography (TLC). The fractions obtained from the methanolic extract ether extracts were subjected to thin-layer chromatography (TLC). Plates (Silica 60 F_{254}, Merck, 250 mm thick) were developed with toluene: ethyl acetate: formic acid (5 : 4 : 1) which separated components into a wide range of R_f values. The components were visualized under visible and UV light (254 and 366 nm) and sprayed with different diagnostic reagents in order to reveal spots of different groups: Dragendorffs reagent for alkaloids, methanolic potassium hydroxide for coumarins, ferric chloride for phenolics, aluminium chloride for flavonoids and anisaldehyde/sulfuric acid for steroids and terpenes. Saponin identification by TLC was done following the reported methodology.[7,8]

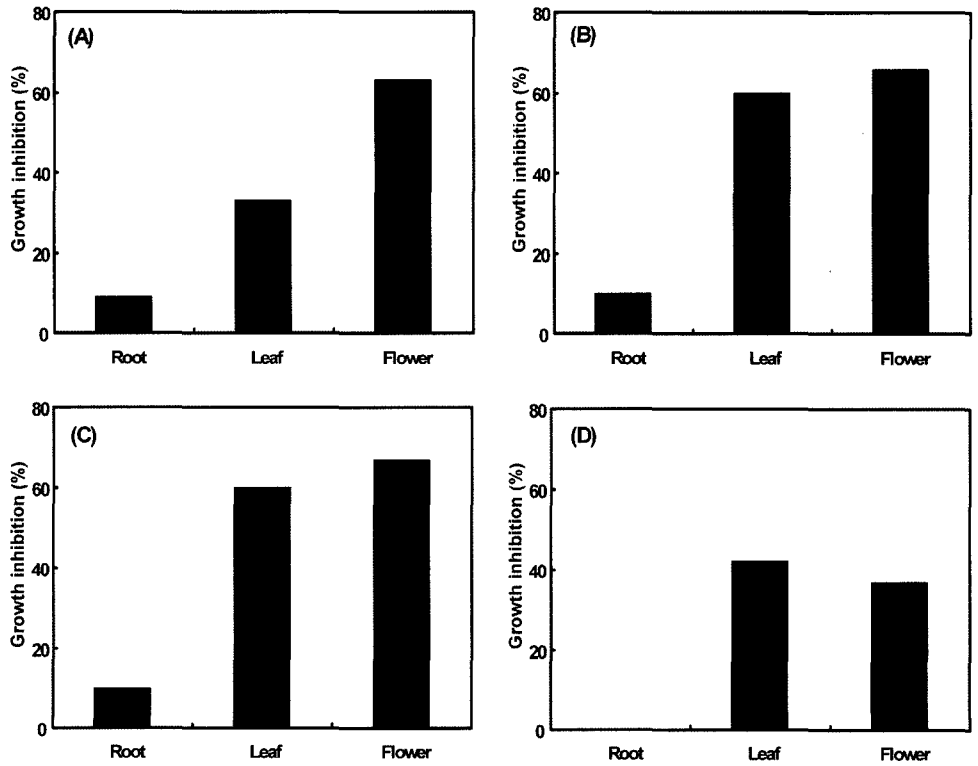

Fig. 2. Effect of *Yucca* methanolic extract of different parts of *Yucca* on the growth of plant pathogens such as *F. oxysporum* (A), *P. capcisi* (B), *R. solani* (C), and *B. cinerea* (D) at concentration of 0.1%.

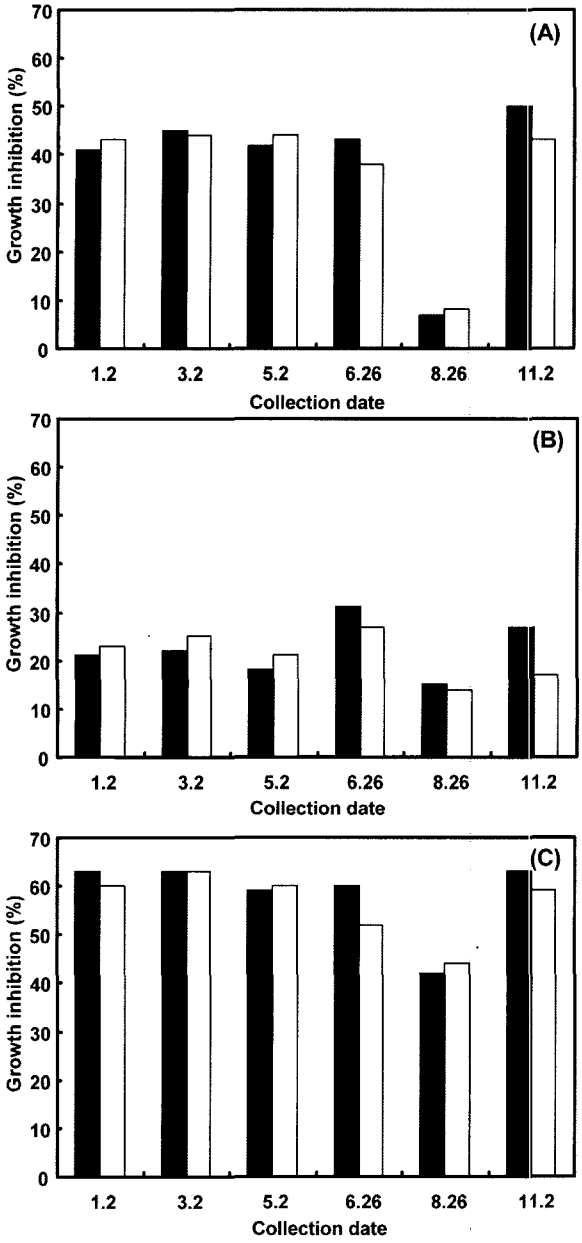

Fig. 3. Collection of *Yucca* leaves and its effect on activity of plant pathogens such as *F. oxysporum* (A), *P. capcisi* (B), and *R. solani* (C). At concentration of 0.1% MeOH extract (■) and the MeOH extract equivalent to1.0% fresh *Yucca* (□).

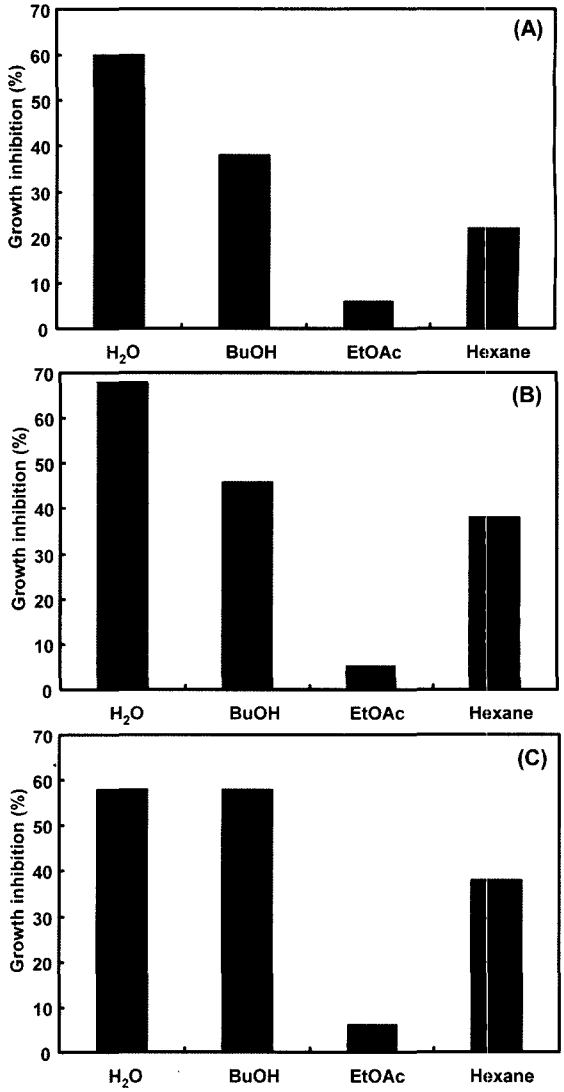

Fig. 4. Effect of each fraction from methanolic extract of *Yucca* leaves on the growth of plant pathogens such as *F. oxysporum* (A), *R. solani* (B), and *B. cinerea* (C).

Results and Discussion

Agar Diffusion Assay. Preliminary liquid culture and agar plate assays showed that the growth of *F. oxysporum*, *P. capcisi*, *R. solani*, and *B. cinerea* was inhibited by *Y. smalliana* extracts (Fig. 1). There was no significant difference in the antifungal activity of the leaf extract of *Y. smalliana* applied before or after sterilization of the growth media. Optimal extract concentration for biological assaying was 0.1%. All following assays were carried out on sterilized media containing extracts of the studied plant.

Measurement of the antifungal activity at different growth stages was made using leaves, (Table 1, Fig. 3). Samples collected on August 26 showed about 45.0% inhibition rate against *R. solani*, 7.0%, inhibition activity against *B. cinerea* and no inhibition against *F. oxysporum*. The samples taken during other seasons showed similar antifungal activity against the tested fungi. Leaves methanolic extracts (0.1 and 1.0%) collected at different periods during plant growth were also tested against *R. solani*, *B. cinerea*, *P. capcisi* and *F. oxysporum*, (Table 1, Fig. 1). Both concentrations showed similar fungicidal activity.

The antifungal activity of the methanolic extracts (1.0%) of the different parts of *Y. smallina* was shown in Fig. 2. The root extract showed no significant activity against *F. oxysporum*, *P. capsici*, *R. solani*, and *B. cinerea*. Flowers and leaves extracts showed antifungal activity of 64.0% and 34.0% against *F. oxysporum*, 66.0% and 62.0% against *P. capsici*, and 27.0% and 41.0% against *B. cinerea*, respectively.

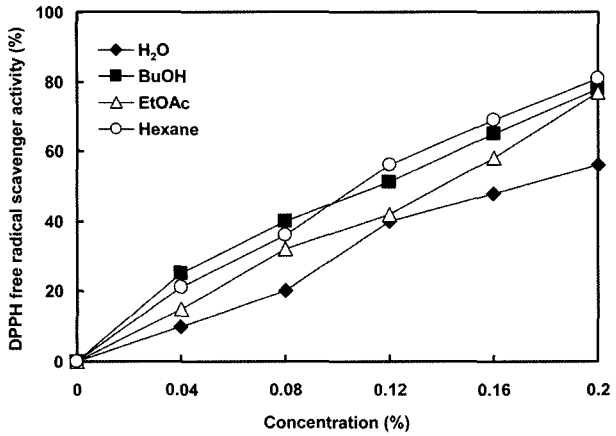

Fig. 5. DPPH free radical scavenger activity of fractions of methanolic extract of *Yucca* leaves. Scavenging activity was measured at 517 nm after 30 min of reaction.

Fractions obtained from the leaves methanolic extract, hexane (12.3 g), ethyl acetate (18.6 g), butanol (100 g), and water (43.9 g), were assessed for their antifungal activity against *F. oxysporum, R. solani, C. gloeosporioides*, and *B. cinerea* at a concentration of 0.1%, (Fig. 4). Strong fungicidal activity was observed from the aqueous and butanol fractions. The aqueous fraction showed inhibition rate of 60.0, 67.8, 84.6 and 58.3% against *F. oxysporum, R. solani, C. gloeosporioides,* and *B. cinerea* respectively, and the butanol fraction showed 36.0, 46.0, 66.1 and 58.3% activity, respectively. On the other hand, the ethyl acetate fraction showed very low antifungal activity of 6.2, 6.2, 35.5, and 6.0%. These results suggest that *Yucca* contains a number of metabolites which contribute to these effects.

Biological control of cucumber damping-off disease. *In vivo* biological control of yucca extracts against cucumber damping-off disease is presented on Fig. 6. Pots inoculated with *R. solani* showed typical damping-off disease symptoms. The affected cucumber plants (35 out of 48 seedlings) were falling down and withering away as they were seriously discolored and constricted on the surface of the ground. However, in pots (48 seedlings) treated with yucca extracts (1.0%), seedlings were growing normally, even though some showed a mild inhibition of growth only for two weeks. These results suggests that the yucca extract can be used as an effective natural pesticide against cucumber damping-off disease caused by *R. solani*, without any side effects on plant growth also without affecting the plant yield.

Biological control of *Phytophthora* blight on red pepper. The effect of yucca extract (1.0%) on red pepper *Phytophthora* blight is shown on Fig. 7. The effect of *Phytophthora* blight inoculation was observed after a growing period of 10 days. Seedlings in pots inoculated with *P. capsici* showed symptoms of the fungus disease. Leaves turned yellow with dark-brown spots on them, and the infected plants developed dark brown decaying spots around their roots. However, in pots treated with yucca extract (1.0%) plus pathogen, no *Phytophthora*

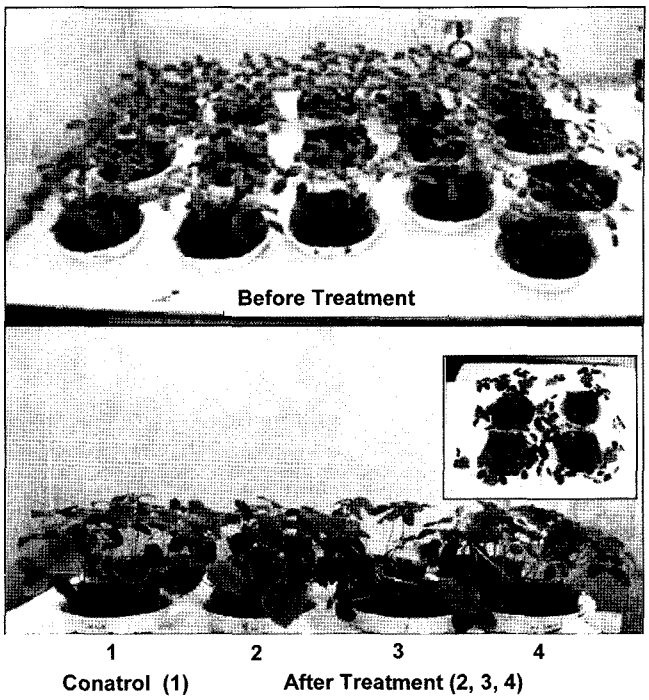

Fig. 6. Control of damping-off caused by *R. solani* on cucumber plants by yucca extract.

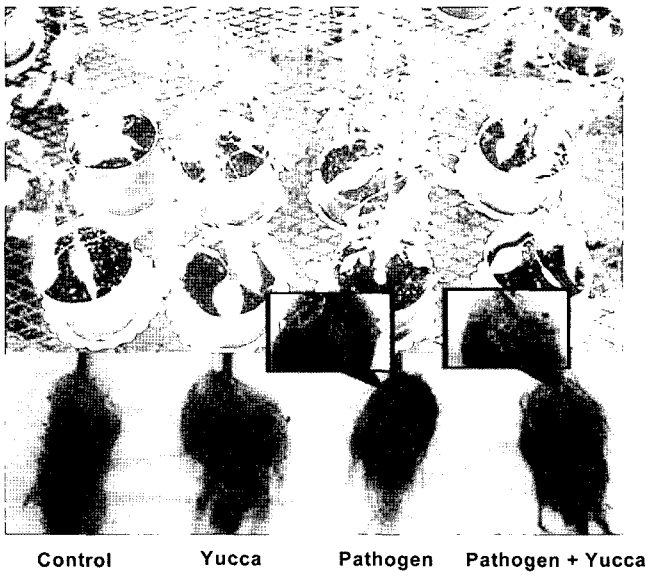

Fig. 7. *In vivo* antifungal activity of yucca extract on the growth of pepper infected by *P. capsici*.

blight was observed, and pots treated only with yucca extract showed no yield loss compared with the untreated pots (Fig. 7). The results suggest that the yucca extract inhibit red-pepper *Phytophthora* blight.

TLC Profile. The TLC pattern revealed the presence of a number of phenolics in the aqueous extracts. Its foaming nature along with the obtained TLC profile suggest the presence of saponins. The chemistry and bioactivity of *Yucca* saponins and phenolics have recently been reviewed by

Table 2. DPPH free radical scavenger activity of the methanolic extract of different parts of *Yucca* organ

Plant part	Concentration (mg/ml)	DPPH reduction (%)
Leaves	1.0	100
	0.5	50.8
	0.2	26.8
	0.1	13.8
Flowers	1.0	37.1
Roots	1.0	0.0

Piacente *et al.*,[9] and the best known are the steroidal saponins. Interactions of saponins with cholesterol and other sterols account for many of their biological effects, particularly those involving membrane activity. Recently it has been recognized that *Yucca* contains other physiologically-active constituents, particularly polyphenols. Of these most important were different types of stilbenes e.g. resveratrol. Stilbenes, such as resveratrol, have been recognized earlier as phytoalexins.[10] Resveratrol was identified previously in grapes and is believed to be a phytoalexin produced by the plant to fight fungal colonization. Also, some unique compounds with *spiro* confirmation were isolated and were given trivial names of yuccaols A-E. Both butanol and ethyl acetate fractions revealed the presence of flavonoids, terpenoids, sterols and saponins (data not shown).

Antioxidant Activity. The antioxidant activities of the extracts of different parts of the plant studied are shown in Table 2. Leaves were most active reducing DPPH by 100% at concentration of 1 mg/ml, flowers showed some slight activity (37.0% DPPH reduction), and the roots had no activity. Both leaves and flowers extracts possessed flavonoids in their TLC profile and these compounds could be responsible for this activity. Flavonoids are famous for their antioxidant activity. Inhibition of DPPH activity by stilbenes has been reported on related bioassays.[11,12] In addition, in these stilbenes the presence or absence of a methoxyl group increases or decreases, respectively the strength of these compounds upon inhibition of DPPH.[2]

Biotic pesticides originated from plants, microorganisms, and animals control certain harmful insects without destroying an ecosystem and harming to human bodies. Despite those advantages, however, they should be developed differently according to the kinds of harmful insects, that means different numbers of harmful insects require the same different numbers of biotic pesticides. This study set out to develop a new antibacterial material from the resource plants easily available around and the strong antibacterial activities of *Y. smalliana* were discovered.

All together, in an attempt to test the feasibility of *Y. smalliana* extracts as a biological pesticide, they were applied to cucumber damping-off and *Phytophthora* blight for the biotic control.[13,14] *Y. smalliana* extracts in this study turned out to have enough controlling effects against the pathogens, *R. solani* and *P. capsici* with few impacts on the plant growth.

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