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Inhibitory effects of *Phytolacca americana* extracts of different solvents on *Pestalotiopsis microspora*: The causal agent of blueberry leaf spot

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Abstract

Inhibitory effects of different *Phytolacca americana* L. extracts on *Pestalotiopsis microspora*, a fungal pathogen isolated from blueberry leaf spot, were determined by a growth rate method. Effects of *P. americana* extracts from different tissues (leaves, roots, stems) obtained with five different solvents on inhibitory rates of *P. microspora* were determined by measuring fungal growth. Ethanol and acetone *P. americana* extracts clearly inhibited growth of the fungal pathogen, and inhibition rate was positively correlated with treatment concentration. Inhibitory effects of ethanol extracts were significantly greater than those of the acetone extracts, and the optimal inhibitory effect (Using EC_{50} to express the median lethal concentration) was at 0.004 g mL^{-1}. Water, petroleum ether, and benzene extracts did not significantly affect *P. microspora* growth. Leaf extracts of *P. americana* had the strongest inhibitory activity, followed by that of root and stem extracts. In this study, the ethanol extract from *P. americana* leaves had the greatest inhibitory effect on *P. microspora*, the causal agent of blueberry leaf spot, and thus, ethanol might be the best choice of solvents to extract bacteriostatic substances from *P. americana*. The study provides basic data for continued research and development of biopesticides.

Introduction

Phytolacca americana is in the family Phytolaccaceae, and it has a wide range of growth, strong adaptability, easy harvest and low price(Zhang *et al.* 2020).In addition to medicinal value(Zou *et al.* 2019), P. americana has attracted much attention for its insecticidal(Wang *et al.* 2019), acaricidal, bacteriostatic, and antiviral activities.

Zhao et al. measured the inhibitory effects of Phytolacca acinosa extract on four types of bacteria by a filter paper method(Zhao *et al.* 2010). In that study, some of the Phytolacca extracts had antibacterial activity, and most of the substances with the strongest antibacterial activity were in the root system. Overall, antibacterial activities of different extracts were different against different bacteria. Ge et al. showed that extracts from fruits and branches of Phytolacca significantly inhibited proliferation of tobacco mosaic

virus(Ge *et al.* 2015). Although bacteriostatic effects of Phytolacca have been reported, bioassays of Phytolacca bacteriostatic activity on pathogenic fungi are lacking.

In this study, extracts were obtained from roots, stems, and leaves of P. americana by using five different solvents: water, petroleum ether, acetone, ethanol, and benzene. Inhibitory effects of the different extracts on the pathogenic fungi P. microspora isolated from blueberry leaf spot were determined. The goal was to clarify differences in the inhibition of P. microspora activity by the different extracts to provide ideas for the development of biopesticides.

Methods

The damaged blueberry leaves were collected from Maling Township Blueberry demonstration Garden, Huaxi District, Guiyang City, Guizhou Province, China (latitude 106.594775, longitude 26.275049). For identification based on ITS(NCBI nucleotide sequences PP346193 and PP346194). The fungal pathogen was identified as *Pestalotiopsis microspora*.

Whole *P. americana* were collected from Guding Village, Xiaba Town, Wudang District, Guiyang City, Guizhou Province, China(latitude 106.898299, longitude 26.692004). Roots, stems, and leaves were dried at 38° C, crushed, and sifted through a 20-mesh screen.

Water, petroleum ether, acetone, ethanol and benzene with different polarity were selected to form a series of solvents. Five concentration gradients were set, and two different extraction methods (Yang *et al.* 2005).

Fragments of the symptomatic leaves were used for indirect isolation of the causal agent in potato dextrose agar (PDA) medium (Jéssica *et al.* 2022). Mycelial disks of Pestalotiopsis microspora was prepared by a growth rate method. And colony diameter was measured by crossing method.

Results

Inhibition rate of acetone extracts from Phytolacca americana against Pestalotiopsis microspora

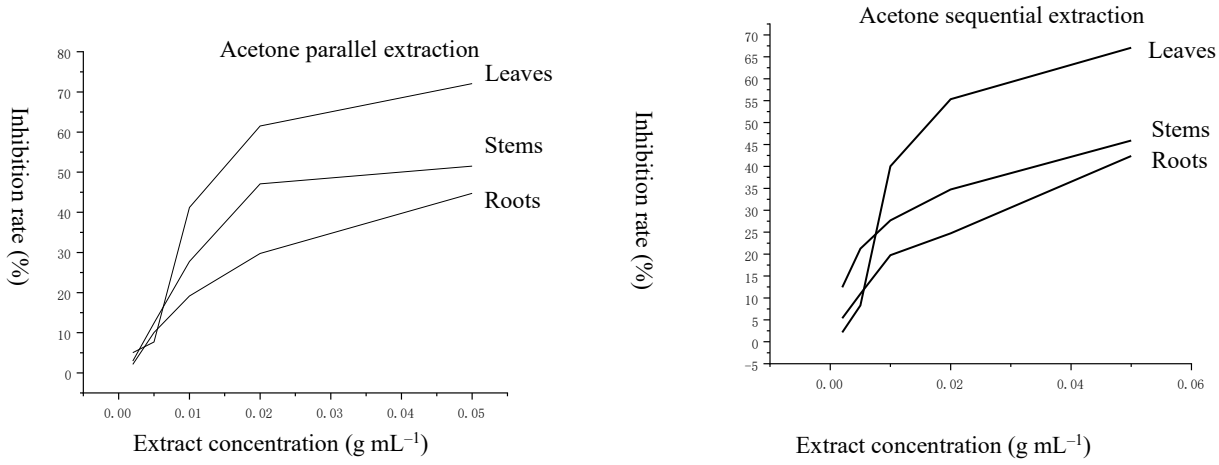

Figure 1 Comparison of inhibitory rate of *Phytolacca americana* acetone extracts from two different extraction methods

Inhibitory rate of acetone extracts from roots, stems, and leaves of *P. americana* on *P. microspora* are given in Figure 1 . When the concentration was 0.05 g mL^{-1} from parallel extraction, the leaf extract had the strongest inhibitory effect, with an inhibition rate of 72.1%. When the concentration was 0.002 g mL^{-1}, the inhibition effect of the root extract was weak, with an inhibition rate of only 2.1%. In sequential extraction, the inhibition rate of the leaf extract at 0.05 g mL^{-1} was 67.1%, which was the maximum inhibition rate of sequential extracts. Acetone extracts of both parallel and sequential extraction methods had inhibitory effects on *P.microspora* across the range of concentrations, with bacteriostatic rate increasing with increasing extract concentration. In parallel and sequential extractions, leaf extracts had the highest inhibitory rate. Overall, treatment concentration and inhibition rate were positively correlated for acetone extracts from different parts of *P. americana*.

Inhibitory rate of ethanol extracts from Phytolacca americana against Pestalotiopsis microspora

Inhibitory rate of ethanol extracts from roots, stems, and leaves of *Phytolacca americana* on *P. microspora* are shown in Figure 2 . In parallel extraction, the strongest inhibition effect was with the leaf extract at 0.05 g mL^{-1} , with the inhibition rate of 77.59%. In sequential extraction, the inhibition rate of the leaf extract at 0.05 g mL^{-1} was 71.99%, which was the maximum inhibition rate of sequential extracts. The inhibition rate of root, stem, and leaf ethanol extracts from parallel extraction increased with the concentration. Overall, treatment concentration and inhibition rate were correlated for ethanol extracts from different parts of *P. americana.*

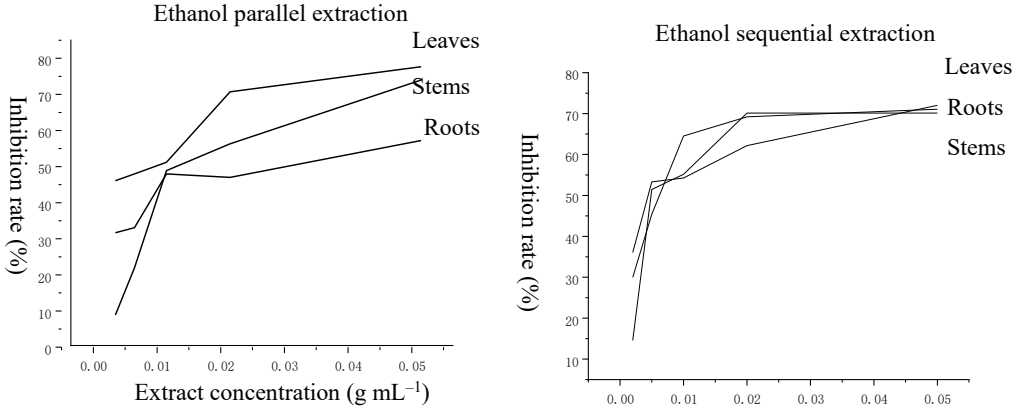

Figure 2 Comparison of inhibitory rates of *Phytolacca americana* ethanol extracts from two different extraction methods

Comparison of virulence and significance analysis of ethanol and acetone extracts of Phytolacca americana against Pestalotiopsis microspora

According to inhibition rates, virulence regression equations were established, and inhibitory medium concentrations (EC_{50}) were calculated. Virulence regression equations and correlation coefficients (r) were used to compare the toxicity of *P. americana* ethanol and acetone extracts to *P. microspora* (Figure 3). according to EC_{50} values of extracts from roots, stems, and leaves, the inhibitory effects of ethanol extracts were generally greater than those of acetone extracts, and the inhibitory effects of leaf extracts were better than those of root and stem extracts.

When acetone was used as the extractant at 0.05 g mL^{-1}, whether parallel or sequential extraction, the inhibitory rate of leaf extracts was significantly higher than that of stem and root extracts. When ethanol was used as the extractant, the inhibitory rate of the leaf extract was significantly higher than that of the stem extract with parallel extraction, but there was no significant difference among root, stem, and leaf extracts when extracted sequentially. Thus, extraction method and order affected the bacteriostatic rate.

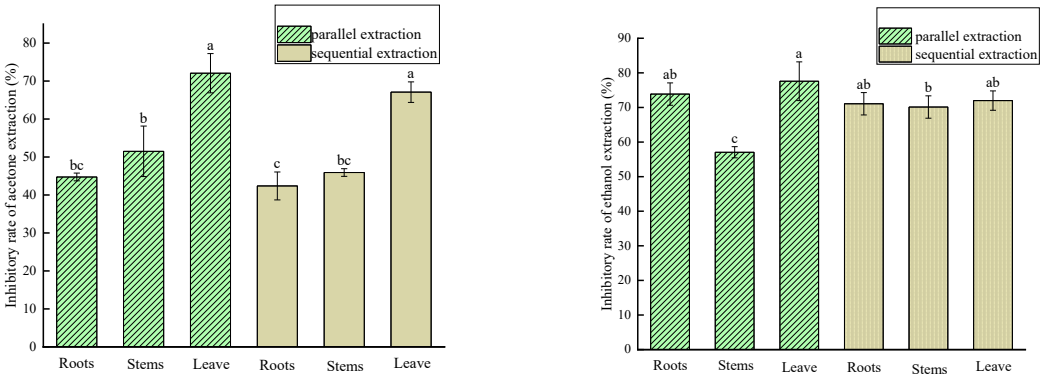

Figure 3 Comparison of inhibition rates of acetone and ethanol extracts of *Phytolacca americana* at 0.05 g mL^{-1} using two extraction methods. Acetone extract on the left and ethanol extract on the right.

Discussion

Secondary metabolites in many plants can inhibit, kill, or promote the growth of pathogenic fungi (Luo *et al.* 2022), and those of *P. americana* also have this characteristic (Li *et al.* 2021). In this study, Both ethanol and acetone extracts inhibited the growth of *P. microspora*, and the concentration of extracts was positively correlated with the inhibition rate. When the concentration of extracts increased, the effective content of the plant extract and the inhibition rate also increased. In addition, analysis of the relation between solvent polarity and bacteriostatic rate indicated that the content of active inhibition components extracted from *P. americana* was positively correlated with solvent polarity. The water extract showed no inhibitory effect, and we thought that the inhibitory components against *P.microspora* were insoluble or slightly soluble in water.

The effective inhibition components of *P. americana* were concentrated in the leaves, which is consistent with the conclusion that most secondary metabolites are primarily concentrated in leaves(Salvat *et al.* 2004). The results are in contrast to those of Zhao *et al.* who found that most substances with the strongest antibacterial activity are in roots, which may be due to Zhao et al.testing effects on bacteria. The inhibition mechanisms of extracts from different parts of *P.americana* against fungi and bacteria might be different and should be investigated in future studies.

Antibacterial substances in different parts of *P.americana* were most easily extracted by strong polar organic solvents.Ethanol extracts of leaves had the highest levels of bacteriostatic active components, which were significantly higher than those of root and stem extracts. To develop plant-derived blueberry leaf spot inhibitors from extracts of *P.americana*, strong polar organic solvents such as ethanol should be used with extraction of leaves.

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References

Ge Y, Luo H, Zheng J, Chen X, Geng Z (2015) Antitobacco mosaic virus activity of triterpenoid saponin from *Phytolacca americana* L. Chinese Journal of Pesticide Science 17, 300-306.

Jéssica MIJ, Rodrigues ABL, Kássia LMP, Cunha MG (2022) *Pestalotiopsis microspora* causes leaf spot on Adeniumobesum. Journal of Phytopathology 6, 170.

Li T, Yin Q, Wu X, Jiang S, Wang Y, Dharmasena DSP, Wei X, Chen Z (2021) Studies on the identification and biology of the pathogenic fungi Pestalotiopsis trachicarpicola of tea leaf spot in Yuqing County. China Plant Protection 41, 24-30+23.

Luo Z, Wei X, Yu L, Li L, Wu Z, Wu D (2022) Screening of antibacterial activity of 10 plant alcohol extracts against wood rot fungi. China Forest Products Industry 59, 46-51.

Salvat A, Antonacci L, Fortunato RH, Suarez EY, Godoy HM (2004) Antimicrobial activity in methanolic extracts of several plant species from northern argentina. Phytomedicine 11(2-3), 230-234.

Wang J, Chen H (2019) Study on insecticidal activity of extracts from root of Phytolacca americana against Apolygus lucorum. Journal of West Anhui University 35, 64-66.

Yang B, Ding W, Zhao Z, Liang Z (2005) Antifungal activities of the extracts from Phytolacca americana and curcumalonga. Journal of SouthWest Agicultural University (Natural Science) 3, 297-300+304.

Zhao GD, Wang LK, Duan J, Mao DZ, Liu Y, Liu H, Meng FG (2010) Analysis the antimicrobial activities of fractions with different polarity, root and stem extracted from Phytolacca acinosa Roxb. Genomics & Applied Biology 29, 717-720.

Zhang, X.Y, Zhang YM, Liu XY, .Zhang CY,Dong SD, Liu Q, Deng M (2020) Cd uptake by Phytolacca americana L. promoted by cornstalk biochar amendments in Cd-contaminated soil. International Journal of Phytoremediation 22, 251-258.

Zou W, Pan Y, Zhang Z, Chen G (2019) Allelopathic effects of extracts of Phytolacca americana on four crops. Journal of Hunan Agricultural University (Natural Sciences) 45, 472-477.