EVALUATION OF THE INHIBITORY ABILITY OF *PIPER NIGRUM* AND *PIPER DIVARICATUM* LEAF WATER EXTRACTS ON *PHYTOPHTHORA CAPSICI* IN *IN-VITRO*

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SUMMARY

Phytophthora capsici is a notorious pathogen of many economically important crops leading to severe impacts on agriculture worldwide. Many plant species are capable of synthesizing compounds that act as a defense against pathogen attacks, showing the potential for application in preventing harmful pathogens on plants. In the current study, we conducted a chemical composition analysis and evaluated the impact of *P. nigrum* and *P. divaricatum* leaf water extracts on the growth of *P. capsici*. GC-MS analysis revealed that spathulenol derivatives (53.64%) were the major components in *P. nigrum* leaf water extract, while eugenol (43.63%) and methyl eugenol (52.24%) were the main components in *P. divaricatum* leaf water extract. Assessing the growth inhibition of *P. capsici* at different concentrations (5 mg/mL, 10 mg/mL, 50 mg/mL) over 72 hours, results showed that *P. divaricatum* leaf water extract (43.02 ± 3.72%) was more effective at inhibiting growth compared to *P. nigrum* leaf water extract (22.18 ± 3.82%). This study opens up prospects for the application of naturally derived anti *P. capsici* compounds in agricultural production.

Keywords: Extract, GC-MS, *in-vitro*, *Phytophthora capsici*, *Piper nigrum*, *Piper divaricatum*.

INTRODUCTION

Phytophthora capsici is an oomycete, Chromista a highly destructive plant pathogen that harms various parts of crops such as roots, stems, fruits, and basal parts (Dunn *et al*., 2014). Previously, *P. capsici* was reported to primarily infect plants in the genus capsicum, such as peppers. However, current reports indicate that this species can affect up to 49 plant families, including tomatoes, eggplants, cucurbits (cucumbers, watermelons, melons, and squashes), as well as some legumes like fava beans, common beans, snap beans, and strawberries (Dunn *et al*., 2014). *P. capsici* can cause up to 100% yield loss once it infects the host under favorable conditions. Temperatures from 25 to 28°C and humidity levels above 80% are considered optimal for the pathogen's infection, development, and disease spread. Moreover, its spores can survive for over 12 months in the absence of a host (Gandariasbeitia *et al.*, 2019). This makes management challenging and positions *P. capsici* as one of the most damaging organisms to crops, significantly affecting agricultural productivity worldwide (Dunn *et al*., 2014).

Managing diseases caused by *P. capsici* is both costly and challenging. Chemical control remains widely used due to its high effectiveness in controlling crop diseases despite numerous issues such as changes in the physicochemical properties of the soil, accumulation of toxic compounds in fruits, and the development of fungicide resistance in pathogen populations (Wan *et al*., 2020). According to Sharma và đồng tác giả (2019), the widespread use of chemical pesticides for controlling agricultural crop diseases has led to a serious global crisis. Chemical pesticides have resulted in many adverse effects, such as changes in soil fertility, loss of biodiversity, nutrient depletion, and damage to the environment and human health (Sharma *et al*., 2019). This is why integrating various control methods such as genetic, breeding, biological, and natural compound approaches is necessary to reduce reliance on chemical pesticides. The use of plant-derived compounds antagonistic to crop pathogens is considered a promising approach to replacing harmful chemical pesticides, reducing economic burdens, and being environmentally friendly.

Plants, having been exposed to pathogens for millions of years, have evolved to synthesize their own compounds for self-protection. These compounds are largely non-toxic to the environment and are mainly known in various forms such as terpenoids, phenylpropanoids, fatty acid derivatives, and amino acids (Cadena *et al*., 2018). Piperaceae is a large family of angiosperms with about 3,600 species divided into five main genera, with *Piper* being the most common. Studies on *Piper* species have shown that, besides their economic utility, these plants possess medicinal properties that have been exploited in traditional medicine since ancient times, including antibacterial, antifungal, and antioxidant capabilities (Bratati *et al*., 2022). *P. nigrum* is widely used as a food in family meals and is a well-known member of this genus. *P. divaricatum*, primarily distributed in Latin American countries, is less known and is often used for bathing in the treatment of rheumatism. Extracts of *P. divaricatum* are believed to contain high amounts of phenylpropanoids, such as methyl eugenol and eugenol, which have

several biological activities (Oliveira *et al*., 2022). Although *P. nigrum* and *P. divaricatum* are valuable economically and medicinally, research on them is increasingly of interest due to the presence of various phytochemicals and their preventive capabilities against pathogens (Bratati *et al*., 2022). In agriculture, research on the preventive capabilities of leaf extracts from Piperaceae species against pathogens such as *P. capsici* remains limited. Therefore, in the current study, we evaluated the inhibitory potential of leaf water extracts from *P. divaricatum* and *P. nigrum* on *P. capsici*.

MATERIALS AND METHODS

Plant materials

P. nigrum was collected in Quang Tri province and *P. divaricatum* was collected in Gia Lai province. Then they were planted in the campus of Institute of Biotechnology, Hue University, (Hue, Vietnam). Plant material was taxonomically identified by the Laboratory Molecular Biology, Institute of Biotechnology, Hue University (Hue, Vietnam) and leaves were collected for experiments.

Extracts preparation

100g of fresh leaves of *P. divaricatum* and *P. nigrum* collected were thoroughly washed in tap water and sterile distilled water. Then the leaf sample was ground using a blender. The paste was added to 1L of sterile distilled water in a beaker, stirred vigorously and left for 1 hour, then filtered through four times of sterile filter cloth to obtain the leaf water extract. Store samples at 4^{o}C.

Testing the composition of the extract by GC-MS assay

The *P. nigrum* and *P. divaricatum* leaf water extract (1ml) after extraction was brought to the Drug, cosmetic and food quality control center of Thua Thien Hue province (HueQC). The extract was checked for composition using a GC-MS system (MS-TQ8040, Shimadzu, Japan). The detected compounds were confirmed through comparison of peak spectra with mass spectra from the library database at HueQC center.

Effect of *P. nigrum* and *P. divaricatum* leaf water extracts on the growth of *P. capsici*

The effect of the extract on the growth of *P. capsci* was measured *in-vitro* using the mycelial growth rate method and was adjusted (Yin *et al*., 2021). Potato Dextrose Agar (PDA) was poured into sterilized Petri dishes and P. *nigrum* and *P. divaricatum* leaf water extracts were added to the PDA medium to achieve the desired concentration of 0 mg/ml (control), 5 mg/mL, 10 mg/mL, 50 mg/mL. A plug of mycelium (5 mm) was taken from the edge of the colony using sterile tweezers and placed in the center of the petri dish with the above mentioned drug. Culture at room temperature for 3 days, measure mycelium length. Each treatment has 3 repetitions. The inhibition effect of the leaf water extract was calculated according to the formula: % inhibition = [(Growth diameter in the control group – Growth diameter in the extract group)/Growth diameter in the control group] x 100 (Al-Hetar *et al*., 2010)

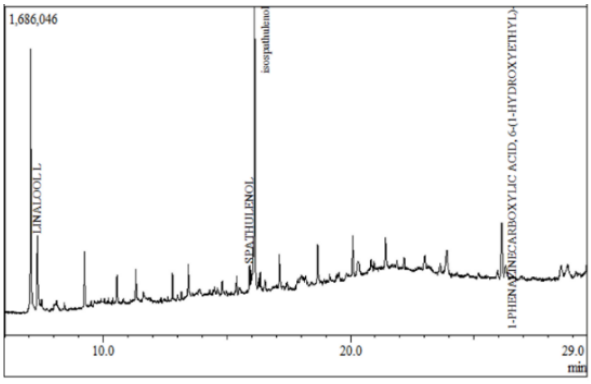
Statistical analysis

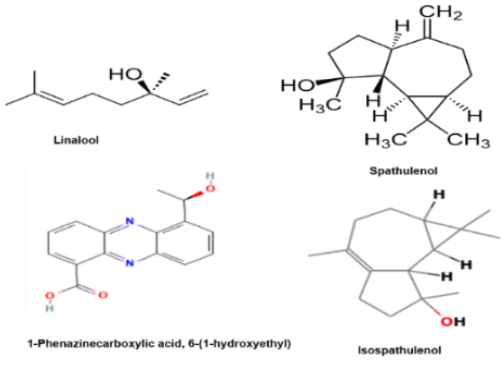
The results of the *P. capsici* resistance survey are given as the mean ± SD of the tested concentrations, each test performed in triplicate. One-way ANOVA was used to determine statistical significance.

RESULT AND DISCUSSION

GC-MS analysis

GC-MS analysis of *P. nigrum* and *P. divaricatum* leaf water extracts from was conducted, identifying and describing 8 compounds with their retention times, molecular weights, formulas, areas, and percentage areas. Each peak suggests the presence of different bioactive compounds in each extract (Figures 1 and 2).

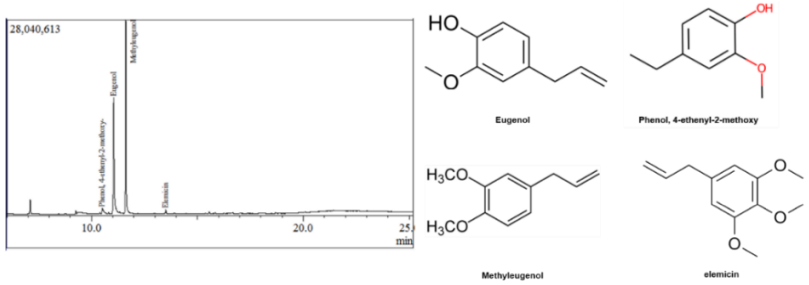




Peak	R. Time	Area	Area%	Name of Compound	Molecular Weight	Molecular Formula
1	7.332	864322	17.94	Linalool	154.25	$C_{10}H_{18}O$
2	15.921	216242	4.49	Spathulenol	220.55	$C_{15}H_{24}O$
3	16.126	2584112	53.64	Isospathulenol	150.17	$C_5H_{24}O$
4	26.122	1153086	23.93	1-Phenazinecarboxylic acid, 6-(1-hydroxyethyl)	286.27	$C_{15}H_{12}N_2O_3$

Figures 1. Results of GC-MS analysis of *P. nigrum* leaf water extract

From the GC-MS analysis of *P. nigrum* leaf water extract, the identified compounds include: linalool (17.94%), spathulenol (4.49%), isospathulenol (53.64%), and 1-Phenazinecarboxylic acid, 6-(1-hydroxyethyl) (23.93%). Similarly, four compounds were identified in the of *P. divaricatum* leaf water extract: Phenol, 4-ethenyl-2-methoxy (3.21%), eugenol (43.63%), methyl eugenol (52.24%), and elemicin (0.92%). The results from Figures 1 and 2 show that spathulenol derivatives are the main components in the *P. nigrum leaf water* extract, while eugenol derivatives are the main components in the *P. divaricatum* leaf water extract. Studies indicate that these chemical constituents exhibit various bioactivities. According to do Nascimento và đồng tác giả (2018), spathulenol exhibits the highest antioxidant activity in DPPH and MDA systems compared to reference standards, with IC_{50} values ranging from 26.13 to 85.60 µg/mL. Additionally, spathulenol (GI_{50} = 49.30 µg/mL) is particularly effective against ovarian cancer cell lines and shows anti-tuberculosis activity against M. tuberculosis with an MIC value of 231.9 µg/mL (Nascimento *et al*., 2018).



Peak	R. Time	Area	Area%	Name of Compound	Molecular Weight	Molecular Formula
1	10.508	3505466	3.21	Phenol, 4-ethenyl-2-methoxy	152,19	$C_9H_{12}O_2$
2	11.047	47604882	43.63	Eugenol	164,20	$C_{10}H_{12}O_2$
3	11.622	56989612	52.24	Methyl eugenol	178.23	$C_{11}H_{14}O_2$
4	13.511	1002328	0.92	elemicin	208.25	$C_{12}H_{16}O_3$

Figures 2. Results of GC-MS analysis of *P. divaricatum leaf water* extract

The *P. divaricatum* leaf water extract is reported to contain high levels of phenylpropanoids, such as methyl eugenol and eugenol, with various biological activities (Oliveira *et al*., 2022). In the context of research and practical applications, plant-derived compounds have been studied and extracted to combat various harmful organisms. Eugenol is one of the promising terpenes for managing biological pests. Eugenol has exhibited interesting biological properties including antibacterial, antioxidant, insecticidal, and other medicinal uses (Nasiou *et al*., 2020). Previous studies have shown that eugenol at a concentration of 660 µg/mL has toxic effects against *M. incognita* (Nasiou *et al*., 2020). Similarly to eugenol, Methyl eugenol has moderate antibacterial activity and acts as a natural anesthetic, capable of inducing rapid and comprehensive immobilization. According to Oliveira và đồng tác giả (2022), extracts from *P. divaricatum* are described as having antioxidant properties, antibacterial activity against both gram-negative and gram-positive bacteria, insecticidal effects, and antifungal properties against plant pathogens (Oliveira *et al*., 2022).

Effect of *P. nigrum* and *P. divaricatum* leaf water extracts on the growth of *P. capsici*

The effects of *P.nigrum* and *P. divaricatum* leaf water extracts on the growth ability of *P. capsici* were analyzed in this study and the results are shown in Figures 3 and 4. Over the observation period of *P. capsici* growth to determine the resistance of the two extracts, we found that both extracts had a positive effect in reducing the growth and development of *P. capsici*. The results were monitored continuously over 3 days at concentrations of 5 mg/mL, 10 mg/mL, and 50 mg/mL.

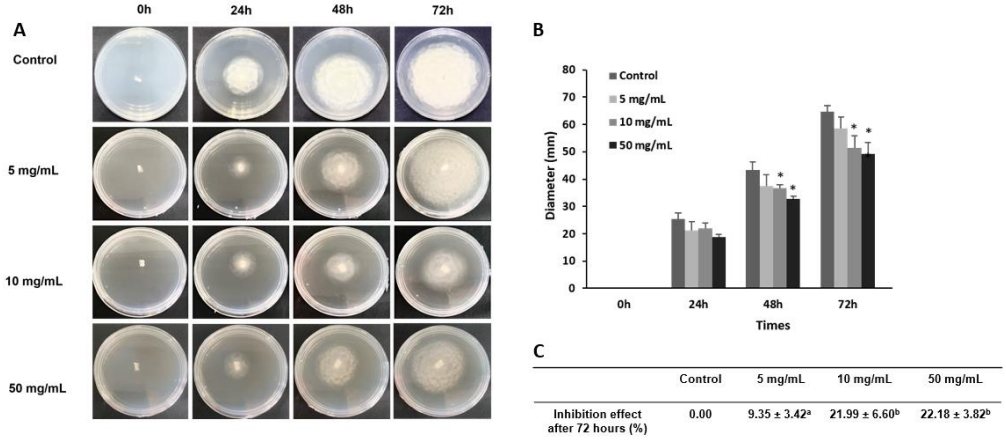


Figure 3. The effect of *P. nigrum* leaf water extract on the growth of *P. capsici*

(A): Morphology of P. capsici after 72 hours of exposure to P. nigrum leaf water extract. (B): growth diameter of P. capsici. (C): Inhibition effect of P. nigrum leaf water extract.

P. nigrum leaf water extract has an effect on the growth rate of *P. capsici* (Figure 3.A). However, the level of impact is not significant. The results showed that after 72 hours the growth diameter of *P. capsici* in the control group (64.67 ± 2.30 mm) and the 5 mg/mL concentration group (58.67 ± 4.16 mm) had no significant difference (Figure 3.B). In contrast, the 10 mg/mL (36.67 ± 1.15 mm) and 50 mg mg/mL (32.67 ± 1.15 mm) groups began to differ from the control group after 48 hours. The inhibitory effect of *P. nigrum* leaf water extract after 72 hours at concentrations of 5 mg/mL, 10 mg/mL, 50 mg/mL was 9.35 ± 3.42%, 21.99 ± 6.60%, 22.18 ± 3.82%, respectively. At two concentrations, 10 mg/mL and 50 mg/mL, there is no significant difference (Figure 3.C).

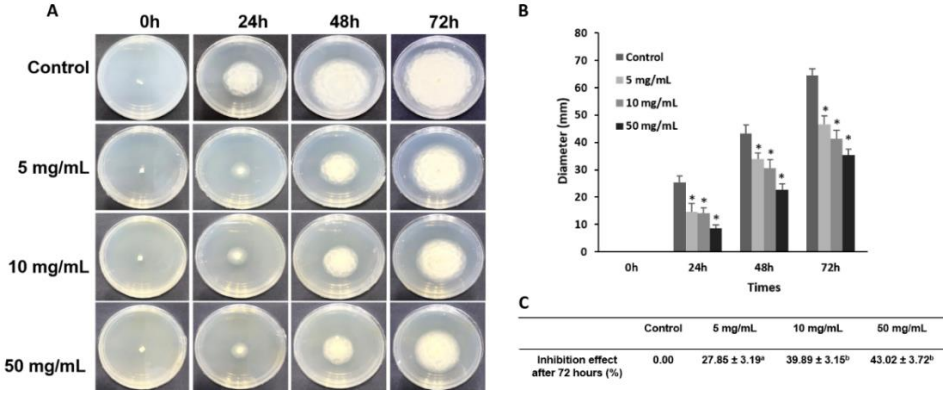


Figure 4. The effect of *P. divaricatum* leaf water extract on the growth of *P. capsici*

(A): Morphology of P. capsici after 72 hours of exposure to P. divaricatum leaf water extract. (B): growth diameter of P. capsici. (C): Inhibition effect of P. divaricatum leaf water extract.

The *P. divaricatum* leaf water extract exhibited a better inhibitory effect on the growth rate of *P. capsici* compared to the *P. nigrum* leaf water extract. Results showed that after 24 hours, there was a significant difference in the growth diameter of *P. capsici* across all concentrations: 5 mg/mL (14.67 ± 3.05 mm), 10 mg/mL (13.34 ± 2.30 mm), and 50 mg/mL (8.66 ± 1.15 mm), compared to the control group (25.34 ± 2.30 mm) and the *P. nigrum* group (Figure 4.A). After 72 hours, *P. divaricatum* leaf water extract exhibited strong resistance to *P. capsici* with growth diameters at 5 mg/mL (46.67 ± 3.05 mm), 10 mg/mL (41.34 ± 3.05 mm), and 50 mg/mL (35.34 ± 2.31 mm), which decreased by 0.27, 0.36, and 0.45 times compared to the control group (64.67 ± 2.30 mm) (Figure 4.B). The inhibition efficiency of *P. divaricatum* leaf water extract after 72 hours at concentrations of 5 mg/mL, 10 mg/mL, and 50 mg/mL was 27.85 ± 3.19%, 39.89 ± 3.15%, and 43.02 ± 3.72%, respectively, higher than *P. nigrum* group (Figure 4.C). According to Truong và đồng tác giả (2023), studies on black pepper varieties resistant to quick wilt (*P. capsici*) and slow decline (*Meloidogyne incognita*) pathogens identified two samples, *P. hancei* (HUIB_PH30) and *P. divaricatum* (HUIB_PD36), with high resistance to all biological stresses (Truong *et al*., 2023). In addition, studies on the ability of *P. divaricatum* to resist plant diseases also show that eugenol and its derivatives are the main components in the plant and have strong resistance activity against pathogens on pepper plants. When seedlings of *P. divaricatum* were introduced into a nursery containing black pepper infected with *Fusarium solani f. sp*. piperis, they exhibited complete and indefinite resistance to the fungus, demonstrating the potential

inhibitory effects of methyl eugenol and eugenol present in P. divaricatum (Da Silva *et al*., 2014). Chen *et al*. (2022) studied 26 derivatives of eugenol as agents against *P. capsici*. Results showed that among all compounds, especially derivatives 3a, 3f, and 3n, exhibited the strongest oomycete inhibitory activity against *P. capsici* with EC_{50} values of 79.05, 75.05, and 70.80, respectively (Chen *et al*., 2022). These findings underscore the potential application of *P. divaricatum* extract in preventing plant pathogens.

Inhibitory effect of *P. nigrum* and *P. divaricatum* leaf water extracts

The inhibitory effect on the growth of *P. capsici* by two extracts, *P. nigrum* and *P. divaricatum*, after 3 days of cultivation, was also compared (Figure 5). It can be observed that both *P. nigrum* and *P. divaricatum* leaf water extracts have an impact on the growth of *P. capsici* on PDA medium, with the best results observed at a concentration of 50 mg/mL. *P. divaricatum* leaf water extract showed better inhibitory effectiveness compared to *P. nigrum* leaf water extract. After 72 hours, there was no significant difference in *P. nigrum* leaf water extract between concentrations of 10 µg/mL (21.99 ± 6.60%) and 50 mg/mL (22.18 ± 3.82%). In contrast, *P. divaricatum* leaf water extract showed a significant difference at all three concentrations: 5 µg/mL (27.85 ± 3.19%), 10 µg/mL (39.89 ± 3.15%), and 50 mg/mL (43.02 ± 3.73%), which were 2.9 times, 1.8 times, and 1.9 times higher, respectively, compared to *P. nigrum* leaf water extract at the same concentrations. The difference in the impact of *P. nigrum* and *P. divaricatum* leaf water extracts on *P. capsici* could be attributed to differences in their chemical compositions. GC-MS analysis of *P. divaricatum* leaf water extract revealed eugenol (43.64%) and methyl eugenol (52.24%) as major components. Studies have consistently shown the strong antibacterial potential of these compounds. Similarly, *P. divaricatum* leaf water extract containing eugenol and methyl eugenol demonstrates potential in inhibiting plant pathogens (Oliveira *et al*., 2022; Nascimento *et al*., 2018; Chen *et al*., 2022). Conversely, although studies have indicated the potential antibacterial and antifungal properties of spathulenol in *P. nigrum* (Nascimento *et al*., 2018), GC-MS analysis showed relatively low levels (4.49%), while isospathulenol, despite its high proportion (53.64%), has limited research on its ability to inhibit plant pathogens.

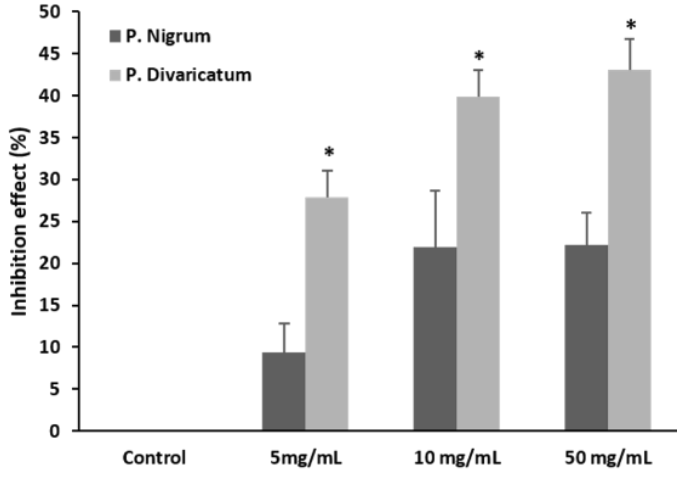


Figure 5. Inhibitory effect of *P. nigrum* and *P. divaricatum* extracts on the growth of *P. capsici*

CONCLUSIONS

This study has demonstrated that leaf water extracts from *P. nigrum* and *P. divaricatum* can affect the growth of *P. capsici*. Specifically, *P. divaricatum* leaf water extract, with chemical components such as eugenol and methyl eugenol, showed a more effective inhibition potential against *P. capsici* compared to *P. nigrum* leaf water extract. These results suggest the potential development of disease control methods against *P. capsici* using naturally sourced compounds, meeting the demand for environmentally friendly disease-resistant compounds in agricultural production.

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ĐÁNH GIÁ KHẢ NĂNG ỨC CHẾ CỦA DỊCH CHIẾT NƯỚC LÁ *PIPER NIGRUM* VÀ *PIPER DIVARICATUM* LÊN *PHYTOPHTHORA CAPSICI* TRONG ĐIỀU KIỆN *IN-VITRO*

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SUMMARY

Phytophthora capsici là một tác nhân gây bệnh nổi tiếng trên nhiều loại cây trồng có giá trị về mặt kinh tế dẫn đến ảnh hưởng nghiêm trọng cho ngành nông nghiệp trên toàn thế giới. Nhiều loài thực vật có khả năng tổng hợp nên các loại hợp chất có vai trò như một biện pháp bảo vệ trước các cuộc tấn công của tác nhân gây bệnh, cho thấy tiềm năng ứng dụng phòng ngừa tác nhân gây hại trên cây trồng. Trong nghiên cứu hiện tại, chúng tôi tiến hành phân tích thành phần hóa học và đánh giá khả năng ảnh hưởng của dịch chiết nước lá *P. nigrum* và *P. divaricatum* lên mức độ sinh trưởng của *P. capsici*. Phân tích GC-MS cho thấy dẫn suất của Spathulenol (53.64%) chiếm thành phần chủ yếu trong dịch chiết nước lá *P. nigrum* còn eugenol (43.63%) và methyl eugenol (52.24%) chiếm thành phần chủ yếu trong dịch chiết nước lá *P. divaricatum*. Đánh giá khả năng ức chế sinh trưởng của *P. capsici* ở các nồng độ khác nhau (5 mg/mL, 10 mg/mL, 50 mg/mL) trong 72 giờ, kết quả cho thấy dịch chiết nước lá *P. divaricatum* (43.02 ± 3.72%) có hiệu quả ức chế tốt hơn so với dịch chiết nước lá *P. nigrum* (22.18 ± 3.82%). Nghiên cứu này mở ra triển vọng cho việc ứng dụng các hoạt chất kháng *P. capsici* có nguồn gốc tự nhiên trong sản xuất nông nghiệp.

Từ khóa: Dịch chiết, GC-MS, *in-vitro*, *Phytophthora capsici, Piper nigrum, Piper divaricatum*.

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