Antifungal activity of mangosteen pericarp and cashew leaf extract against *Fusarium oxysporum in vitro*

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ABSTRACT

Polyphenols are secondary compounds that occur widely in plants and are highly effective in controlling plant pathogenic microorganisms. This study aimed to screen polyphenolic-rich plant extracts for their antifungal potential against *Fusarium oxysporum*. Several plant materials including cashew leaves, castor fruits, castor leaves, coffee husks, giant milkweed leaves, mangosteen pericarps and soapberry fruits were investigated for their total phenolic content. The results showed that cashew leaves and mangosteen pericarps contained high level of polyphenols at 108.23 and 124.14 mg GAE/g, respectively. The main phenolic compounds found in cashew leaves were gallic acid and protocatechuic acid at 377.29 mg/100 g and 56.44 mg/100 g, respectively. Mangosteen pericarps contained 16.22 mg/100 g protocatechuic acid and 55.75 mg/100 g of chlorogenic acid. The antifungal activity of cashew leaf and mangosteen pericarp extracts against *F. oxysporum* was 32.92 - 77.08% and 68.33 - 83.75%, respectively at the extract concentration from 2% to 10%. The combined use of cashew leaf and mangosteen pericarp extracts exhibited an additive inhibition against *F. oxysporum*. Cashew leaves and mangosteen pericarp are potential materials for producing bio-fungicides, which are not only effective but also safe for human and the environment.

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1. Introduction

Fusarium oxysporum is a soil-borne pathogen which causes *Fusarium* wilt disease on many plant species such as banana, tomato, peas and seriously affects crop yield by up to 80 - 90% (Ma et al., 2013; Edel-Hermann & Lecomte, 2019). Management of *Fusarium* wilt disease has been a challenge for many years. Controlling plant fungal diseases using chemical pesticides is not a sustainable solution because of high cost, increasing resistant strains, residual toxicity in the food chains and the environment. Biological control agents have gained much attention as alternatives to synthetic chemicals due to their effectiveness and safety (Muller-Riebau et al., 1995). The use of crude plant extracts rich in bioactive compounds has been approached and applied widely in agricultural processes. Polyphenols are secondary metabolites occurring abundant in plants with antioxidant, antibacterial and antifungal activities, which exhibit effective inhibition against fungal pathogens (Yuan et al., 2018). Phenolic compounds affect mycelial growth by inhibiting spore reproduction, disrupting cell membranes, and suppressing the synthesis of important proteins or enzymes (Oufensou et al., 2020). In our previous study, antifungal activity was directly proportional to the content of phenolic compounds in the plant extracts (Nguyen et al., 2024). Among locally available plant materials, cashew leaf and mangosteen pericarp emerged as prominent phenolic-rich sources, which were investigated for their main constituents and inhibitory effect on the growth of *Fusarium oxysporum*. The study provided useful information for further research and development of polyphenol-derived bioproducts for effective control of *Fusarium oxysporum*.

2. Materials and Methods

2.1. Plant materials, chemicals and reagents

Raw materials including cashew leaves (*Anacardium occidentale* L.), castor fruits and leaves (*Ricinus communis* L.), coffee husks (*Coffea arabica* L.), giant milkweed leaves (*Calotropis gigantea* L.), mangosteen pericarps (*Garcinia mangostana* L.) and soapberry fruits (*Sapindus saponaria* L.) were collected in Ho Chi Minh City, Binh Phuoc and Lam Dong province. The sample was washed, drained naturally at room temperature then dried at 50°C until the moisture content reached below 13%. Then, they were pulverized into fine powder, sieved through a sifter with a pore size of 0.1 mm and stored in zipper bags in desiccators at room temperature. The fungal pathogen *F. oxysporum* f. sp. *lycopersici* was provided by the Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City.

Gallic acid, chlorogenic acid and protocatechuic acid were purchased from Sigma-Aldrich. Acetonitrile and acetic acid (HPLC grade) were supplied by Thermo Fisher Scientific. Folin-Ciocalteau's reagent was provided by Supelco. All other chemicals were of analytical grade.

2.2. Determination total phenolic content

Plant extracts were prepared by mixing 1 g of plant material with 70% aqueous ethanol with a ratio of raw material to solvent of 1:10. The extraction was conducted using ultrasonication with a frequency of 20 kHz and power of 500 W for 15 min. Then, the extract was obtained by filtering through filter papers (Whatman No.1). The residual solid was extracted with 70% aqueous ethanol two more times. The extracts were combined and made up to a total volume of 50 mL.

Total phenolic content (TPC) was determined by spectrophotometry according to the reaction with Folin-Ciocalteu reagent (Trinh et al., 2018). Briefly, 100 µL of the prepared extract was mixed with 100 µL of Folin-Ciocalteu reagent. After 5 min, 300 µL of 20% Na_2CO_3 solution was added to the mixture to stop the reaction prior to making up to 5 mL with distilled water. The mixture was incubated for 60 min in darkness at room temperature. Its absorbance was measured at 730 nm. Gallic acid was used as the standard with a concentration range of 100 - 500 mg/L. TPC was expressed as milligram gallic acid equivalent per gram of dry matter (mg GAE/g).

2.3. Quantification of phenolic compounds in plant extracts

Gallic acid (GA), protocatechuic acid (PCA) and chlorogenic acid (CGA) in the extracts were determined by high performance liquid chromatography (HPLC) with a diode array detector (Agilent 1260 Infinity II, Santa Clara, United States). A binary pump was set up with mobile phase A (1% acetic acid in water) and mobile phase B (1% acetic acid in acetonitrile). A Poroshell 120-EC C18 reversed-phase column (100 mm × 4.6 mm, particle size 2.7 µm) was used for separation at a column temperature of 35°C. The mobile phase and elution program are presented in Table 1. The sample injection volume was 5 µL and the mobile phase was injected at a flow rate of 1 mL/min. Gallic acid and protocatechuic acid were detected at 270 nm; chlorogenic acid was detected at 320 nm (Trinh et al., 2018). Results were expressed in mg/100 g of dry material.

Table 1. Elution program for separation of phenolic compounds

Time (min)	% A	% B
2	95	5
17	60	40
20	60	40
21	95	5
25	95	5

2.4. Inhibitory activity of plant extracts against *F. oxysporum in vitro*

Fusarium oxysporum was inoculated on potato dextrose agar (PDA) at 28 ± 2°C for seven days. Then, a piece of mycelium with diameter 8 mm was placed on petri dishes (90 × 15 mm) containing PDA medium and plant extracts. Plant extracts at concentrations of 4, 8, 12, 16, 20% were prepared by extracting 4, 8, 12, 16, 20 g of dried plant material, respectively with 70% ethanol using the procedure mentioned earlier (section 2.2), then ethanol was removed by an evaporator followed by the addition of distilled water to make a total volume of 100 mL. The plant extracts were mixed with 2X PDA medium at the same volume to obtain a medium containing 2, 4, 6, 8, 10% plant extracts, respectively. The extract combination of cashew leaves and mangosteen pericarps at 4% and 8% was prepared using 2% and 4% of each, respectively by the same procedure used for the individual extracts.

Post-inoculation plates were incubated at 28 ± 2°C. Mycelium growth was observed at day 1, 3, 5, and 7 after inoculation. Each treatment was conducted in triplicate. The ability to inhibit fungi was evaluated by the following formula (Chang et al., 2000):

$$I = \frac{(D_c - \mathrm{D})}{D_c} \times 100$$

Whereas I is antifungal index, D is diameter of mycelium treated with the extracts, D_c is mycelial diameter of the control without treated.

2.5. Observation of mycelial morphology

In this study, the morphological characteristics of *F. oxysporum* mycelium were observed by microscopic examination. A small piece of the *F. oxysporum* was carefully collected and placed on a clean microscope slide. Then, a drop of sterile saline solution was added to the specimen prior to covering it with a coverslip. Observations were conducted using a microscope (Evident CX23) equipped with 40X objectives (Buffi et al., 2023).

2.6. Data analysis

Experimental results were expressed as mean ± standard error. One-way analysis of variance and Tukey's tests were used to determine significant differences ($P < 0.05$) between the means using the MiniTab 16.0 program.

3. Results and Discussion

3.1. Determination of total phenolic content

This study collected and screened locally valuable plant materials with the aim to search phenolic-rich sources, which are believed to perform antifungal capacity. The total phenolic content of collected samples was found in a wide range of 7.45 - 124.14 mg GAE/g, indicating the differences in the quantity of bioactive compounds in plants (Table 2). Most notably, cashew leaf and mangosteen pericarp contained the highest phenolic levels at 108.23 and 124.14 mg GAE/g, respectively, which were much higher than other samples.

Table 2. Total phenolic content in plant extracts

Plant materials	Total phenolic content (mg GAE/g)
Coffee husks	10.06 ± 0.78
Soapberry fruits	7.45 ± 0.40
Mangosteen pericarps	124.14 ± 0.49
Castor fruits	14.78 ± 0.51
Castor leaves	14.02 ± 0.10
Giant milkweed leaves	10.08 ± 0.22
Cashew leaves	108.23 ± 0.85

Mangosteen pericarps have long been used as a traditional folk medicine for sprains, typhoid, diarrhea and skin infections. It has attracted much attentions due to exhibiting anti-inflammatory, antioxidant, antibacterial and antifungal activity (Kaur et al., 2020). Mangosteen pericarp accounts for about 65% of the total fresh weight of the fruit, with a TPC of 140.66 mg GAE/g containing many substances such as xanthones, phenolic acids, flavonoids, and benzophenones (Rizaldy et al., 2021). Vo & Nguyen (2023) reported that mangosteen pericarp collected in Vietnam had

high polyphenol content at 195.05 mg GAE/g and showed antioxidant activity, inhibitory effect on α-glucosidase enzyme and antibacterial capacity against *Propionibacterium acnes*. Do et al. (2011) isolated α-mangostin and γ-mangostin from the alcohol extract of mangosteen pericarp, which exhibited antioxidant activity and antibacterial effect on *Edwardsiella tarda*. Cashew leaves have shown the ability to inhibit pathogenic microorganisms such as *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Candida albicans,* and *Aspergillus niger* (Shiekh et al., 2022). Duangjan et al. (2019) reported TPC of cashew leaves of 160.35 mg GAE/g with the presence of flavonoids, tannins and anthocyanins. In this study, mangosteen pericarps and cashew leaves, which were the most abundant phenolic sources, were investigated for their antifungal activity and specific phenolic compounds.

3.2. Antifungal activity

Phenolic compounds are the most diverse phytochemicals presenting in plants that have been reported to show biological effects, such as antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory activities (Nguyen et al., 2019, Soyel et al., 2022; Wang et al., 2022). A strong correlation between total phenolic content and antimicrobial activity has been demonstrated (Bouslamti et al., 2022). In our previous study, antifungal activity of plant extracts against *Fusarium oxysporum* was observed to enhance with an increase in the content of phenolic compounds (Nguyen et al., 2024). Figure 1 and Figure 2 show the effect of cashew leaf and mangosteen pericarp extracts on the growth of *F. oxysporum*. The average diameter of fungal colonies ranging from 53.67 to 18.33 mm corresponding to antifungal index ranging from 32.92 to 77.08% was observed on PDA medium supplemented with cashew leaf extracts at 2 - 10% (material weight/total volume). The mycelium didn't spread normally in the medium containing the cashew leaf extracts as compared to the control. A decrease in fungal biomass was observed at all extract concentrations and purple color completely disappeared at 8% cashew leaf extract. The antifungal activity of mangosteen pericarp extract against *F. oxysporum* was higher than that of cashew leaf extract with values ranging from 68.33 to 83.75% at concentrations of 2 - 10% (w/v). Mycelia didn't spread onto the medium supplemented with mangosteen pericarp extracts and the particular purple color wasn't observed at all mangosteen pericarp extract levels investigated.

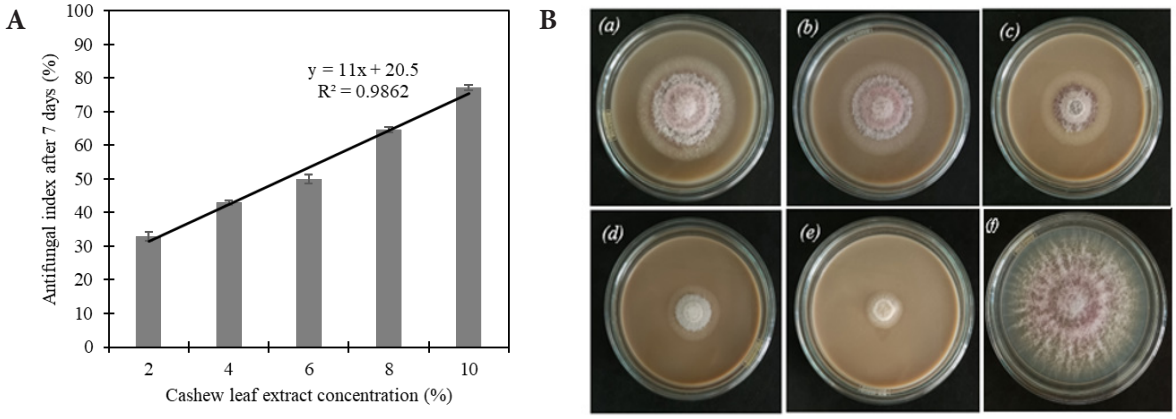

Figure 1. Effect of cashew leaf extracts on *F. oxysporum*. A: Antifungal index; B: Mycelial growth on the media supplemented with the extract at different concentrations (a-f) 2, 4, 6, 8, 10% and control, respectively.

Yenjit et al. (2007) reported that the crude extract of mangosteen pericarp at a concentration of 1000 µg/mL showed high inhibitory effects against *Pythium aphanidermatum, Puccinia psidii, Colletotrichum gloeosporioides,* and *F. oxysporum,* with an antifungal index of 45%, 56%, 34.45%, 34.38%, and 26.88%, respectively. The substances isolated from mangosteen pericarps, such as xanthones, flavonoids, benzophenones, lactones and phenolic acids have been shown to inhibit certain fungal desease in plants. Previous research showed that the aqueous and ethanolic extract of cashew leaf had inhibitory effect on *Aspergillus niger* and the antifungal activity of the extracts increased with increasing extract concentration from 20 - 100 mg/mL. The aqueous extract had a higher fungal inhibition efficacy than the ethanolic extract which might be due to the high concentration of tannins in the aqueous extract (Tafinta et al., 2020).

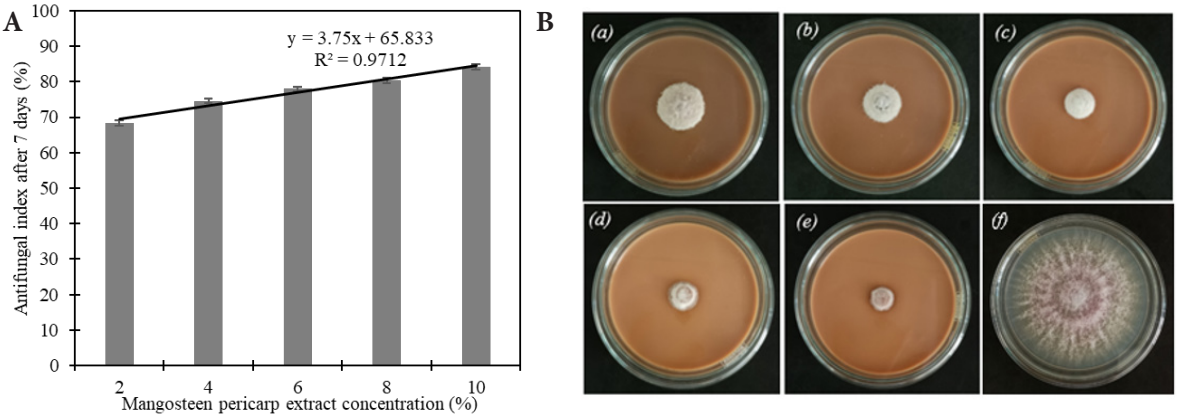

Figure 2. Effect of mangosteen pericarp extracts on *F. oxysporum.* A: Antifungal index; B: Mycelial growth on the media supplemented with the extract at different concentrations (a-f) 2, 4, 6, 8, 10% and control, respectively.

Table 3. Antifungal activity and phenolic content of individual and combined treatment

Treatment	Phenolic content (mg/disc)	Antifungal index (%)
C2	$43.29^{f} \pm 0.34$	$32.92^{e} \pm 1.44$
M2	$49.65^{e} \pm 0.20$	$68.33^{c} \pm 0.72$
C2 + M2	$92.95^{c} \pm 0.52$	$75.00^{b} \pm 0.00$
C4	$86.59^{d} \pm 0.68$	$42.92^{d} \pm 0.72$
M4	$99.31^{b} \pm 0.39$	$74.58^{b} \pm 0.72$
C4 + M4	$185.90^{a} \pm 1.03$	$81.25^{a} \pm 0.00$

C2, C4: Cashew leaf 2%, 4%; M2, M4: Mangosteen pericarp 2%, 4%.

An significant improvement in the inhibitory effect on *F. oxysporum* was reported in our previous study when using a combination of phenolic-rich extracts (Nguyen et al., 2024). In this study, a mixture of cashew leaf and mangosteen pericarp extract was prepared to investigate its effect on *F. oxysporum* in comparison to the individual extract. As displayed in the Table 3, the antifungal activity of the combination of 2% of each extract reached 75%, which was

2.3 and 1.1 folds higher than that of individual cashew leaf and mangosteen pericarp extract, respectively. Similarly, the antifungal activity reached 81.25% when the two extracts were combined at 4% of each, while individual cashew leaf and mangosteen pericarp extract inhibited the mycelial growth by only 42.92% and 74.58%, respectively. It is attributed to the higher phenolic content in the combined treatment compared to the single extract. However, mangosteen pericarp at 2% (M2) showed higher inhibitory effect than cashew leaf 4% (C4) even though it had lower phenolic content, indicating the antifungal activity was not completely propotional to the phenolic content. The combined extract (C2 + M2) showed antifungal activity comparable to a 4% mangosteen pericarp extract (M4), despite having a lower phenolic level, which indeed suggests the role of specific bioactive compounds in the mangosteen pericarp (Table 3). Briefly, mangosteen pericarp demonstrated higher antifungal activity than cashew leaf, and their combination showed an additive effect on mycelial growth of *F. oxysporum*. Our results were in agreement with the publised data. The additive or synergistic effect of extract combination appeared due to the presence of diverse phenolic compounds, such as phenolic acids and flavonoids from different plant species, and each molecule affected *F. oxysporum* by different mechanism of action (Mirghani, 2022; Nguyen et al., 2024).

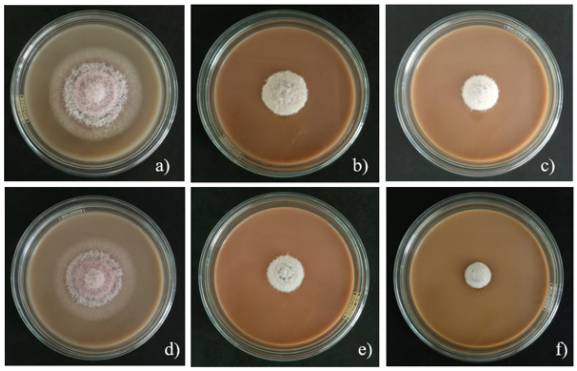

Figure 3. Mycelial growth on the media supplemented with the combined extract at different concentrations: a) C2, b) M2, c) M2 + C2 , d) C4, e) M4 and f) M4 +C4.

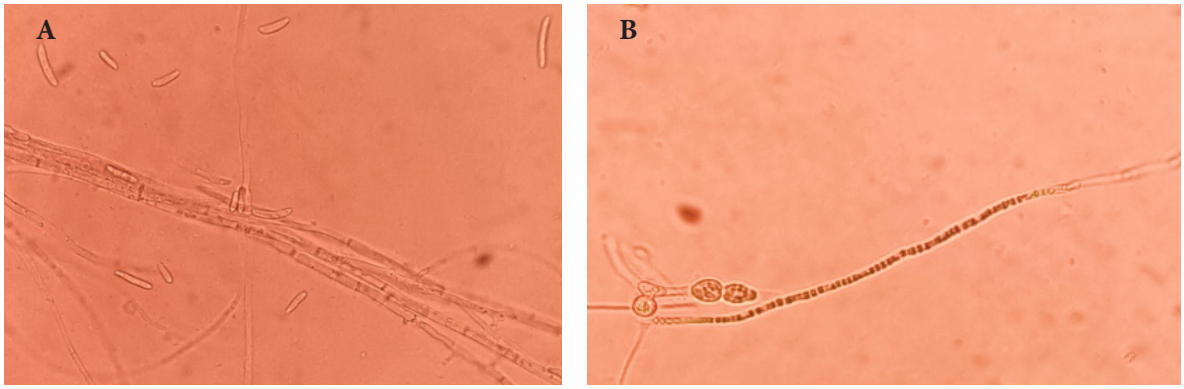

Figure 4. Mycelial morphology under the microscope (40X). A: Without treated; B: Treated with the mixture of 2% cashew leaf and 2% mangosteen pericarp.

The use of extract combination to inhibit fungal growth has been reported in the literature. The mixture of *Lawsonia alba* leaf extract and *Acacia catechu* stem extract showed an increase in inhibitory effect on the *Fusarium solani* growth up to 78.64% compared to that of the individual extracts of 62.07% and 54.69%, respectively (Bhardwaj, 2012). This is attributed to the higher levels of functional compounds in the extract combination than that of individual extracts; the greater diversity of different groups in the mixture and the synergistic effect of specific compounds in the extract combination (Nguyen et al., 2024).

Mycelia on PDA medium supplemented with the combined extract did not spread, underdeveloped, and lost purple pigment (Figure 3). Microscopic mycelial morphology observed on the medium supplemented with the combined extract showed fungal unusual growth including poor formation of hyphal septa and spores compared to the control mycelia. In addition, the intracellular components of mycelial hyphae appeared condensed and different from the control (Figure 4). The inhibitory effect of plant extracts on conidial germination of *F. oxysporum* has been well documented (Mohamed et al., 20217; Alotibi et al., 2020). Moreover, phenolic compounds such as gallic, cinnamic, vanillic, coumaric, ferulic and salicylic acids were demonstrated to induce the destruction and shrunken of fungal hyphae (Wu et al., 2014). In a earlier study, *F. oxysporum* hyphae-treated with *Rumex* sp. extract displayed significant ultrastructural changes, including cell wall deformation and damage, as well as the appearance of electron-dense material along the hyphae (Alotibi et al., 2020). Phenolic compounds with many hydroxyl groups in their structure that are able to bind to adhesives and proteins on membranes, inactivating enzymes, breaking cell membranes, and leaking intracellular substances (Cowan, 1999). Additionally, polyphenols have inhibitory effects on DNA/RNA/protein synthesis and mitochondrial dysfunction (Khanzada et al., 2021). Mode of action includes induction of programmed cell death, suppression of biofilm formation and mycelial growth, and inhibition of soluble protein synthesis that causes increased permeability membrane and disrupts membrane integrity (Acheuk et al., 2022). Therefore, particular phenolic compounds in the mangosteen pericarp and cashew leaf extract have been determined.

3.3. Quantification of specific polyphenols in the extracts

Gallic acid, protocatechuic acid and chlorogenic acid are common phenolic compounds identified in the mangosteen pericarp and cashew leaf. As shown in Figure 5 and Table 4, mangosteen pericarp contained chlorogenic acid and protocatechuic acid at 55.75 mg/100 g and 16.22 mg/100 g, respectively. Cashew leaf was a rich source of gallic acid at 377.29 mg/100 g and protocatechuic acid at 56.44 mg/100 g. Chlorogenic acid is the ester of caffeic acid and quinic acid, a hydroxycinnamic acid synthesized by plants through the phenylpropanoid pathway. According to previous research, chlorogenic acid inhibited spore reproduction and limited mycelial growth of *Fusarium solani, Verticillium dahliae, Botrytis cinerea* and *Cercospora sojina* (Martínez et al., 2017). Gallic acid and its derivatives including pyrogallic acid and syringic acid inhibited the mycelial radial growth of *Alternaria solani* and efficiently suppressed the development of early blight disease without any phytotoxic symptoms on treated tomato plants (El-Nagar et al., 2020). Protocatechuic acid is a widely distributed bioactive compound

found in many plant species. Protocatechuic acid isolated from *Paenibacillus elgii* displayed potent antifungal activity against *Botrytis cinerea* and *Rhizoctonia solani*. Moreover, gray mold formation on strawberry fruit was almost inhibited by protocatechuic acid after 7 days infected with *B. cinerea* conidia (Nguyen et al., 2015). Using the extract mixture containing abundantly diverse phenolic substances to enhance the antifungal activity could be an efficient and sustainable strategy to control plant pathogens and maintain the ecosystem as well.

Table 4. Quantitative analysis of phenolic compounds in plant extracts

Compounds	Gallic acid (mg/100 g)	Protocatechuic acid (mg/100 g)	Chlorogenic acid (mg/100 g)
Mangosteen pericarps	-	16.22 ± 0.39	55.75 ± 0.93
Cashew leaves	377.29 ± 2.05	56.44 ± 1.23	-

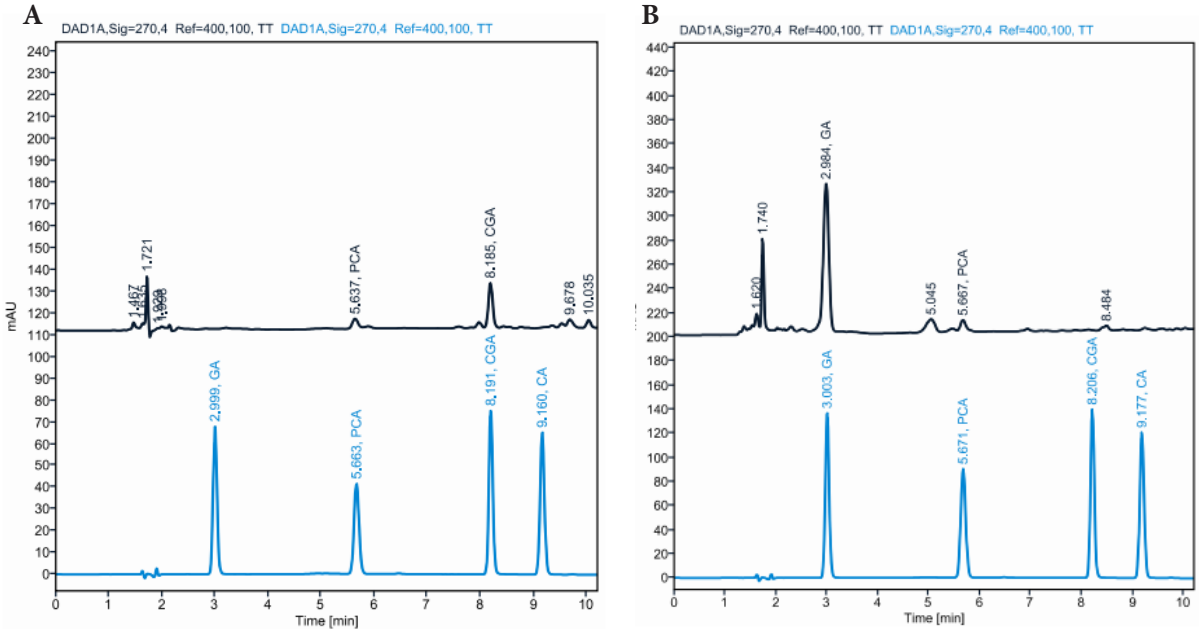

Figure 5. HPLC chromatogram A: Cashew leaves; B: Mangosteen pericarps.
GA: Gallic acid; PCA: Protocatechuic acid; CGA: Chlorogenic acid; CA: caffeic acid
Blue line: chromatogram of the standard mixture.

4. Conclusions

Cashew leaves and mangosteen pericarps contained prominent phenolic levels at 108.23 mg GAE/g and 124.14 mg GAE/g, respectively. Inhibition effects of cashew leaf and mangosteen pericarp extract on the mycelial growth of *F. oxysporum* were 32.92 - 77.08% and 68.33 - 83.75%, respectively at extract concentrations from 2% to 10%. Mangosteen pericarp exhibited greater antifungal activity than cashew leaf, and their combination yielded an additive effect on *F. oxysporum*. Cashew leaf contained 377.29 mg/100 g gallic acid and 56.44 mg/100 g protocatechuic acid; while chlorogenic acid and protocatechuic acid were determined in the mangosteen pericarp at 55.75 mg/100 g and 16.22 mg/100 g, respectively.

Conflict of interest

None of the authors of this study have any financial interest or conflict with industries or parties.

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