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Anti-Fungal Activity of *Moutan cortex* Extracts against Rice Sheath Blight (*Rhizoctonia solani*) and Its Action on the Pathogen's Cell Membrane

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Cite This: *ACS Omega* 2022, 7, 47048−47055

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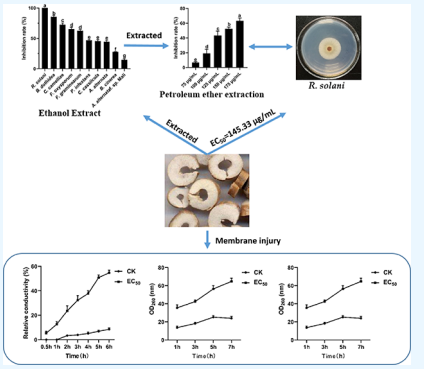
ABSTRACT: Rice sheath blight (RSB) caused by *Rhizoctonia solani* is one of the most destructive diseases of rice (*Oryza sativa*). Although chemical fungicides are the most important control methods, their long-term unreasonable application has brought about problems such as environmental pollution, food risks, and non-target poisoning. Therefore, considering the extraction of fungistatic substances from plants may be an alternative in the future. In this study, we found that the *Moutan cortex* ethanol extract has excellent antifungal activity against *R. solani*, with a 100% inhibition rate at 1000 μg/mL, which aroused our great exploration interest. In-depth exploration found that the antifungal active ingredients of *M. cortex* were mainly concentrated in the petroleum ether extract of the *M. cortex* ethanol extract, which still maintained a 100% inhibition rate with 250 μg/mL, and its effective medium concentration (EC_{50}) was 145.33 μg/mL against *R. solani*. Through the measurement of extracellular relative conductivity and OD_{260}, the petroleum ether extract induced leakage of intracellular electrolytes and nucleic acids, indicating that the cell membrane was ruined. Therefore, we preliminarily determined that the cell membrane may be the target of the petroleum ether extract. Moreover, we found that petroleum ether extract reduced the content of ergosterol, a component of the cell membrane, which may be one of the reasons for the cell membrane destruction. Furthermore, the increase of MDA content would lead to membrane lipid peroxidation, further aggravating membrane damage, resulting in increased membrane permeability. Also, the destruction of the cell membrane was observed by the phenomenon of the mycelium being transparent and broken. In conclusion, this is the first report of the *M. cortex* petroleum ether extract exhibiting excellent antifungal activity against *R. solani*. The effect of the *M. cortex* petroleum ether extract on *R. solani* may be on the cell membrane, inducing the disorder of intracellular substances and metabolism, which may be one of the antifungal mechanisms against *R. solani*.



INTRODUCTION

Rice sheath blight (RSB) caused by *Rhizoctonia solani* is one of the most destructive diseases of rice (*Oryza sativa*) in the world.[1] RSB occurs in the entire growth period of rice, mainly damaging leaf sheaths and leaves.[2] RSB has a strong epidemic and a wide range of infection, which can even reduce rice production by 50% in China.[3] *R. solani*'s sclerotia can resist more extreme environments, which results in difficulties in prevention and control, and has generated a lot of impact on rice's high yield and stable production.[4,5] Due to the lack of resistant varieties, the control of RSB mainly relies on chemical fungicides such as hexaconazole and thifluzamide.[6−8] Among them, chemical fungicides targeting succinate dehydrogenase (SDH) have been widely used due to their superior control prevention and have long been the main control agents in rice planting areas in China.[9] However, due to the single site of action of the target, SDH inhibitor (SDHI) fungicides have a greater risk of resistance. The Fungicide Resistance Action Committee (FRAC) classifies SDHIs as medium to high risk of resistance, and more than 10 *Rhizoctonia* pathogens have developed resistance.[10] This situation will greatly limit the development of SDHI fungicides, and it is urgent to look for new fungicides to control RSB.

Abundant plant resources and their diverse secondary metabolites are valuable resource pools for pesticide leaders.[11] Compared with chemical pesticides, botanical pesticides often have the advantages of novel and diverse targets, low toxicity, and degradability.[12,13] In recent years, various plant extracts

Received: September 23, 2022
Accepted: November 25, 2022
Published: December 7, 2022

have been reported to exhibit excellent antifungal activity against *R. solani*. For instance, the extracts of total tannins from *Larrea tridentata* and *Flourensia cernua* had a 100% inhibition rate on *R. solani* at doses of 2000 and 1000 μg/mL, respectively, and the effective medium concentration (EC_{50}) of the extract of total tannins from *L. tridentata* was 185 μg/mL.[14] The EC_{50} value of the methanol extract of the root from *Pueraria peduncularis* against *R. solani* was 324.72 μg/mL.[15] The leaf extract of *Datura metel* against *R. solani* at a 3.5% concentration caused a 75% retardation in test fungal growth.[16] The acetonic extract of the leaves of *Solanum lycopersicum* showed antifungal activity against *R. solani*, with a minimum inhibitory concentration (MIC) of less than 310 μg/mL.[17] In the meantime, studies have revealed that most plant extracts can destroy the cell structure, inhibit energy metabolism, interfere with substance synthesis, etc., which shows the multi-target features.[18−20] Therefore, the effective substance of the extract from the plant against RSB will replace chemical fungicides and meet the urgent needs of developing green pesticides.

Moutan cortex is used as a Chinese herbal medicine in China, with anti-inflammatory, antioxidant, and other pharmacological activities.[21−23] Many biologically active metabolites, including terpenoids, flavonoids, and phenolic substances, have been found in *M. cortex*, and a special pharmacological role is played by these metabolites.[24,25] In recent years, the antimicrobial and insecticidal activity of *M. cortex* has been gradually reported, such as against yeast, *Aspergillus flavus*, and *Tribolium castaneum*.[26−28] Although *M. cortex* had an antimicrobial activity against microorganisms, it mostly focused on medical microorganisms and food microorganisms, and there were few reports on plant pathogenic microorganisms, especially plant pathogenic fungi.

Therefore, in this study, our group took 10 kinds of phytopathogenic fungi as the research objects and we found that the ethanol extract from *M. cortex* showed strong antifungal activity against *R. solani*. In order to further clarify the active substances and mechanisms of the extract from *M. cortex* inhibiting *R. solani*, ethyl acetate, *n*-butanol, petroleum ether, and water were selected to extract the ethanol extract of *M. cortex*. The destructive effect of the extract of *M. cortex* with the concentration at EC_{50} on the cell membrane was explored. We hope that the *M. cortex* extract will alleviate the resistance pressure of chemical fungicides and provide support for the development of new fungicides against RSB.

MATERIALS AND METHODS

Materials. Pathogenic fungi *Botryosphaeria dothidea*, *R. solani*, *Fusarium graminearum*, *Fusarium oxysporum*, *Colletotrichum camelliae*, *Phytophthora infestans*, *Alternaria alternata*, *Corynespora cassiicola*, *Botrytis cinerea*, and *Alternaria alternata* f. sp. Mali were supplied by the Plant Protection Laboratory of College of Life Science and Agriculture from Qiannan Normal University for Nationalities. Ethanol, petroleum ether, ethyl acetate, *n*-butanol, and biochemical materials were all of analytical reagent grade and purchased from commercial companies. *M. cortex* was purchased from a Chinese herbal medicine market in Chengdu, China. *M. cortex* was pulverized with a pulverizer, passed through a 40-mesh sieve to remove impurities, and soon afterward stored in a sealed bag in a cool place for standby.

Ethanol Extract from *M. cortex*. The ethanol extract of *M. cortex* was obtained by the cold soaking method described in previous studies, with minor modifications.[29] 200 g of *M. cortex* was weighed and put into a wild-mouth bottle, and 2000 mL of 95% ethanol was added. It was subsequently soaked for 48 h at room temperature with manual stirring three times a day. After soaking for 48 h, the liquid was filtered with a Buchner funnel, extracted three times repeatedly, and combined three times with filtrates. The filtrates were concentrated at 45 °C with a rotary evaporator, the obtained extract was weighed, and the extraction rate was calculated and stored in a 4 °C refrigerator for future use.

Antifungal Activity of the Ethanol Extract. The antifungal activity of the ethanol extract from *M. cortex* was detected by calculating the mycelial growth rate.[30] First, the obtained ethanolic extract was dissolved in DMSO to prepare a 250 mg/mL stock solution. 0.2 mL of the stock solution was taken and added to 49.8 mL of potato dextrose agar (PDA) medium to prepare a final concentration at 1000 μg/mL, and the PDA medium without the ethanol extract served as the control. After mixing, it was poured into three sterilized petri dishes (9 cm in diameter). After solidification, the newly activated 0.7 cm disk was inoculated into the petri dish, cultured upside down at 28 °C, and the diameter of the mycelium was measured by the cross method when the mycelium grew to two-thirds of the size of the petri dish. Each treatment was replicated three times. The mycelial growth inhibition rate is calculated according to the following formula:

$$\text{inhibitory rates (\%)} = (\text{Cd(cm)} - \text{Td(cm)})/(\text{Cd(cm)} - 0.07\text{(cm)}) \times 100$$

Among them, Cd represents the mycelium diameter length of the control group and Td represents the mycelium diameter length of the treatment group.

Antifungal Activity of the Extracts of the Ethanol Extract. The petroleum ether, ethyl acetate, and *n*-butanol extracts of the ethanol extract from *M. cortex* were prepared by liquid−liquid extraction, with minor modifications.[31] 25 g of the ethanol extract of *M. cortex* was suspended in 500 mL of water and then extracted with petroleum ether, ethyl acetate, and *n*-butanol for three, four, and five times, respectively. The color of the thin-layer silica gel plate was used as the basis for whether the extraction was complete. The filtrates were collected from each extraction solution through a Buchner funnel and concentrated under reduced pressure using a rotary evaporator to obtain the petroleum ether, ethyl acetate, *n*-butanol, and aqueous extracts, respectively. Two concentrations were established for each extract, 500 and 250 μg/mL; those without the extracts as a control. Each treatment was repeated three times for antifungal activity detection.

Toxicity of the Petroleum Ether Extract from *M. cortex* against *R. solani*. The petroleum ether extract was formulated into the PDA medium with concentrations of 175, 150, 125, 100, and 75 μg/mL; the medium without the petroleum ether extract served as the control. The EC_{50} value was calculated by DPS 7.05 software according to the fungicidal rate from the different concentrations.[32]

Morphological Observation of the Petroleum Ether Extract on *R. solani*. Five 0.7 cm *R. solani* disks were placed in 50 mL potato dextrose broth (PDB) medium and cultured at 28 °C, 160 r/min, for 48 h. Then, the petroleum ether extract was added to make the final concentration of the EC_{50} value and culture was continued at 28 °C, 160 r/min, for 24 h. The medium without the petroleum ether extract served as a

https://doi.org/10.1021/acsomega.2c06150
ACS Omega 2022, 7, 47048−47055

control. The mycelium was taken out, washed two to three times with PBS (pH 7.2) buffer, and stained with cotton blue solution. The mycelium was observed under an optical microscope.[33]

Effect of the Petroleum Ether Extract on Electrical Conductivity on *R. solani*. 0.5 g of the mycelium was weighed and put into a conical flask containing 50 mL of PBS (pH 7.2) buffer and then added with the petroleum ether extract to make the final concentration as the EC_{50} value, and that without the petroleum ether extract served as a control. At this time, the conductivity was measured immediately and recorded as *J*0. Then, they were each processed for 0.5, 1, 2, 3, 4, 5, and 6 h and their electrical conductivity *J*1 was measured in turn. Finally, the mycelium was boiled for 15 min for dead treatment and the conductivity *J*2 was measured.[34] The formula for calculating the relative conductivity at a certain time is as follows:

$$\text{relative conductivity}\ (\%) = (J1 - J0)/(J2 - J0) \times 100.$$

Effect of the Petroleum Ether Extract on OD_{260} on *R. solani*. The treatment method of the mycelium is the same as above. Then, they were each treated for 2, 4, 6, and 8 h. The extracellular suspension was centrifuged at 5000 r/min for 10 min, and the OD value of the supernatant was measured at 260 nm with an ultra-micro spectrophotometer.[35]

Influence of the Petroleum Ether Extract on Ergosterol of *R. solani*. The treatment method of the mycelium is the same as above. The petroleum ether extract was added to make the final concentration of the EC_{50} value, and culture was continued at 28 °C, 160 r/min, for 24 h. That without the petroleum ether extract served as a control. The mycelium was taken out and washed two to three times with PBS (pH 7.2) buffer. The method of ergosterol measurement is described in literature published previously.[36]

Effect of the Petroleum Ether Extract on Malondialdehyde (MDA) on *R. solani*. The treatment method of the mycelium is the same as above. Then, they were each treated for 2, 4, 6, and 8 h. 0.5 g of the mycelium was weighed and ground into a homogenate on ice then centrifuged at 12000 r/min for 10 min. 1 mL of the supernatant after centrifugation was taken, added with 1 mL of 0.6% TBA, and they were mixed together and boiled for 15 min. Then, they were quickly cooled and centrifuged at 4000 r/min for 10 min. Finally, the OD values were measured at 450, 532, and 600 nm.[37]

Statistical Analysis. Both the mean value and the standard deviation (SD) value were calculated by Excel 2016, and the significance ($P < 0.05$) was tested by DPS 7.05 Duncan's new multiple-range method.[32]

RESULTS

Extraction Rate of the Ethanol Extract. After 200 g of *M. cortex* was extracted with 95% ethanol for 48 h, leach liquor was concentrated by a rotary evaporator and 35.61 g of the extract was obtained. As shown in Table 1, the extraction rate was 17.81%.

Table 1. The Extraction Rate of the Ethanol Extract from *M. cortex*

plant	dry powder (g)	extract (g)	extraction rate (%)
M. cortex	200	35.61	17.81

Antifungal Activity of the Ethanol Extract. The antifungal activity of the ethanol extract against the 10 phytopathogenic fungi at a concentration of 1000 μg/mL is shown in Figure 1. From the figure, the ethanol extract showed

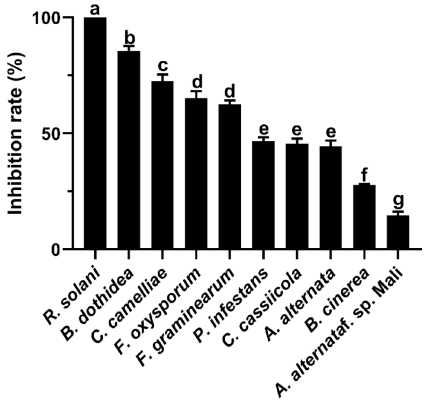


Figure 1. Antifungal activity of the ethanol extract of *M. cortex* at 1000 μg/mL. Different lowercase letters indicate significant differences ($p < 0.05$).

antifungal activity against all the pathogenic fungi. The antifungal activity of *B. dothidea*, *R. solani*, *C. camelliae*, *F. graminearum*, and *F. oxysporum*, with the antifungal rate exceeded 60%, was higher than that of *P. infestans*, *A. alternata*, and *C. cassiicola*. In particular, the activity against *R. solani* was 100%, showing unique antifungal characteristics. However, it showed a weak antifungal effect on *B. cinerea* and *A. alternataf.* sp. Mali.

Antifungal Activity of the Extracts of the Ethanolic Extract against *R. solani*. The antifungal activities of the petroleum ether, ethyl acetate, *n*-butanol, and aqueous extracts of the ethanol extract against *R. solani* are shown in Table 2. It

Table 2. Antifungal Activity of the Extracts of the Ethanol Extract against *R. solani*[a]

extracts	concentration (μg/mL)	inhibition rate (%)
petroleum ether extract	250	100 ± 0a
	500	100 ± 0a
ethyl acetate extract	250	11.34 ± 0.05c
	500	34.42 ± 0.02b
n-butanol extract	250	
	500	
aqueous extract	250	
	500	

[a]The inhibition rate (%) is expressed as "mean ± standard deviation (SD)"; blank cell means no antifungal activity; lowercase letters mean significant $p < 0.05$.

is shown in the table that the petroleum ether extract has the best antifungal activity and the inhibition rate against *R. solani* is 100% at two concentrations of 500 and 250 μg/mL. The ethyl acetate extract showed weak antifungal activity against *R. solani* at the concentrations of 500 and 250 μg/mL, and the inhibition rates were 34.42 ± 0.02 and 11.34 ± 0.05%, respectively. However, the *n*-butanol extract and the aqueous extract had no antifungal activity. Therefore, the petroleum ether extract had the most prominent antifungal activity on *R. solani*, indicating that the antifungal active substances are mainly distributed in the petroleum ether extract.

https://doi.org/10.1021/acsomega.2c06150
ACS Omega 2022, 7, 47048−47055

The Toxicity of the Petroleum Ether Extract against *R. solani*. As shown in Figure 2, the inhibitory rates of the

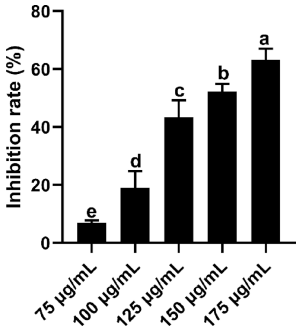


Figure 2. Antifungal activity of the petroleum ether extract of *M. cortex* against *R. solani*. Different lowercase letters indicate significant differences ($p < 0.05$).

petroleum ether extract against *R. solani* were 6.95 ± 0.82, 19.05 ± 5.72, 43.39 ± 5.88, 52.25 ± 2.65, and 63.24 ± 3.76%, with the concentrations of 75, 100, 125, 150, and 175 μg/mL, respectively. It indicated a good linear relationship between the inhibitory concentration and the antifungal activity. Further, we calculated the EC_{50} value to be 145.33 μg/mL, showing excellent fungicidal activity of the petroleum ether extract on *R. solani*.

Effect of the Petroleum Ether Extract on Morphology of *R. solani*. As shown in Figure 3, the mycelium of the

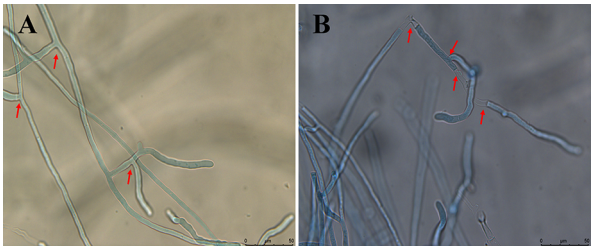


Figure 3. Effect of the petroleum ether extract of *M. cortex* on mycelium morphology. A was treated without the petroleum ether extract of *M. cortex*; B was treated with the petroleum ether extract of *M. cortex* with the EC_{50} value (145.33 μg/mL).

control group had a regular shape and uniform structure and had the basic characteristics of *R. solani*, that is, the mature branch and the new branch were generally at the right angle (Figure 3A). However, after treatment with the petroleum ether extract with the EC_{50} value, the mycelium appeared broken and transparent (Figure 3B). In addition, the angle of the mycelium changed, that is, the branch of the mycelium was no longer at a right angle, indicating that the elasticity of the mycelium had changed. The results showed that the petroleum ether extract caused changes in mycelial morphology of *R. solani*.

Influence of the Petroleum Ether Extract on Electrical Conductivity of *R. solani*. As shown in Figure 4, the relative conductivity of the control group increased slightly within 0.5−6 h. However, the treatment group increased rapidly at EC_{50}, exceeding 50% within 5 h. The results showed that the petroleum ether extract had a damaging effect on the cell membrane of *R. solani*, resulting in increased cell membrane permeability and electrolyte leakage.

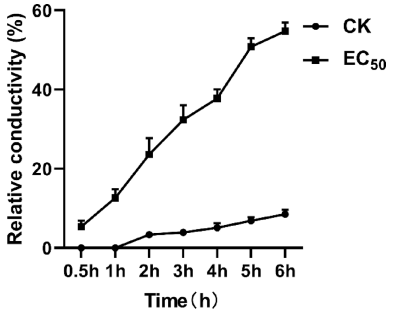


Figure 4. Effect of the petroleum ether extract of *M. cortex* on electrical conductivity.

CK was treated without the petroleum ether extract of *M. cortex*; EC_{50} was treated with the petroleum ether extract of *M. cortex* at 145.33 μg/mL.

Influence of the Petroleum Ether Extract on OD_{260} of *R. solani*. As shown in Figure 5, although the OD_{260} value of

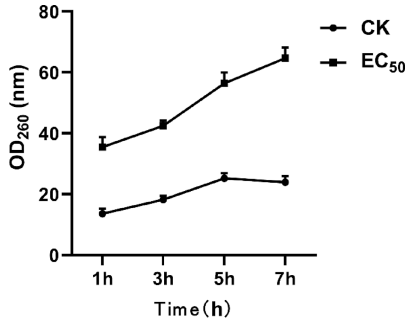


Figure 5. Effects of the petroleum ether extract of *M. cortex* on OD_{260}. CK was treated without the petroleum ether extract of *M. cortex*; EC_{50} was treated with the petroleum ether extract of *M. cortex* at 145.33 μg/mL.

the control group increased, it was very slow. After treatment with the petroleum ether extract with EC_{50}, the OD_{260} value showed a continuous upward trend with time. At 1, 3, 5, and 7 h, the OD_{260} values of the treatment group were 2.60 times, 2.33 times, 2.24 times, and 2.70 times, respectively. The results showed that the petroleum ether extract caused the nucleic acid of *R. solani* to leak out of the cell, and the integrity of the cell was destroyed.

Influence of the Petroleum Ether Extract on Ergosterol of *R. solani*. As shown in Figure 6, after

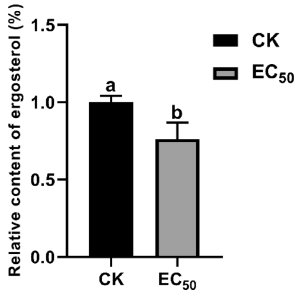


Figure 6. Effects of the petroleum ether extract of *M. cortex* on ergosterol. CK was treated without the petroleum ether extract of *M. cortex*; EC_{50} was treated with the petroleum ether extract of *M. cortex* at 145.33 μg/mL.

https://doi.org/10.1021/acsomega.2c06150
ACS Omega 2022, 7, 47048−47055

treatment with the petroleum ether extract at EC_{50} for 24 h, the content of ergosterol decreased to 76.26% in the control group. The results showed that the petroleum ether extract inhibited ergosterol biosynthesis.

Influence of the Petroleum Ether Extract on MDA of *R. solani*. As shown in Figure 7, the content of MDA in the control group increased slowly with time. Under the EC_{50} of the treatment group, the content of MDA increased sharply with the increase of time. At 1, 3, 5, and 7 h, the MDA contents of the treatment group were 4.67 times, 6.2 times, 3.55 times, and 4.12 times that of the control group, respectively. The results showed that the petroleum ether extract caused membrane lipid peroxidation of *R. solani*, causing membrane damage and aggravating membrane permeability.

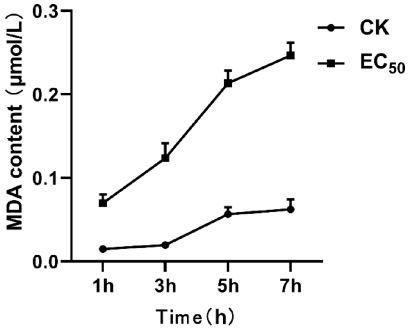


Figure 7. Effect of the petroleum ether extract of *M. cortex* on MDA. CK was treated without the petroleum ether extract of *M. cortex*; EC_{50} was treated with the petroleum ether extract of *M. cortex* at 145.33 μg/mL.

DISCUSSION

Fungi are one of the most important biological factors that harm plants, and the resulting plant diseases pose a serious threat to the health and functioning of natural and manmade ecosystems.[38] Although chemical fungicides are the most important control method, their long-term unreasonable application has brought about problems such as environmental pollution, food risks, and non-target poisoning.[39−41] Therefore, considering the extraction of fungistatic substances from plants may be an alternative in the future.[42,43]

To begin with, the level of the extraction rate directly determined whether the source of active substances is abundant or not, because this will be one of the factors comprehensively considered in the later stage of the development and utilization of fungicides. Moreover, studies have shown that extraction time and the type of organic solvent have an effect on the extraction rate.[44] Chun et al. used 300 g of *M. cortex* with 1000 mL methanol at room temperature for 24 h; the yield was 13.2%.[45] Rho et al. also used 300 g *M. cortex*, but they took 70, 85, and 100% graded ethanol for 10 min ultrasonic treatment and finally mixed them together for suction filtration concentration; the weight of the extract was 62.8 g, and the yield was 20.93%.[46] In this study, 35.61 g of the extract was obtained by concentrating from 200 g of ethanol extraction filtrate of *M. cortex* at room temperature for 48 h, and the extraction rate was 17.81%. Thus, it can be seen that our method adopted ethanol extraction and the yield was high without additional equipment and special handling, which can meet the needs of subsequent experiments.

Through the antifungal activity of the ethanol extract against the 10 phytopathogenic fungi, it was found that the antifungal rate of *B. dothidea*, *R. solani*, *C. camelliae*, *F. graminearum*, and *F. oxysporum* was more than 60%, indicating that the ethanol extract of *M. cortex* had broad-spectrum antifungal activity and exhibited more specific activity against *R. solani* with the inhibition rate of 100% at a concentration of 1000 μg/mL. However, the ethanol extract of *M. cortex* showed weak antifungal activity against *A. alternata* f. sp. Mali, *B. cinerea*, and *A. alternata*. The results revealed that the antifungal activity of the ethanol extract of *M. cortex* had genus selectivity and had a high antifungal effect on *Rhizoctonia*, *Fusarium*, *Colletotrichum*, and *Botryosphaeria*, but the antifungal effect on *Phytophthora*, *Alternaria*, and *Botrytis* was poor. We speculated that the reason for this difference may be the different contents of the cell wall or cell membrane structural components in different species, leading to the different difficulties of antifungal drugs entering the cells, for example, *Phytophthora*, most of which are ergosterol-deficient, so they are less sensitive to agents targeting cell membranes.[47] Compared with other reported extracts of plants, the ethanol extract of *M. cortex* had a better inhibition rate against *R. solani* than the extracts from *Larrea tridentata* (100% at 2000 μg/mL) and *Ageratum conyzoides* ethanol extract (below 70% at 1000 μg/mL).[14,48] Therefore, it is necessary to further extract and narrow the scope of antifungal active substances of *M. cortex*.

Next, we further tested the antifungal activities of the petroleum ether, ethyl acetate, *n*-butanol, and aqueous extracts of the ethanol extract from *M. cortex*. The results showed that the *n*-butanol and aqueous extracts did not show antifungal activity and the ethyl acetate extract had antifungal activity, but the activity was weak. However, the antifungal activity of the petroleum ether extract was particularly outstanding, with 100% at both 500 and 250 μg/mL concentrations. Moreover, the EC_{50} value of the petroleum ether extract against *R. solani* was 145.33 μg/mL, which is better than the methanol extracts from *Pueraria peduncularis* (EC_{50}, 324.72 μg/mL)[15] and the *n*-butanol extract of *Melaleuca alternifolia* (EC_{50}, 780.23 μg/mL),[49] even better than isolated monomeric compounds such as 8-hydroxy-9, 10-diisobutyloxythymol (EC_{50}, 157 μg/mL)[50] and gingerol (EC_{50}, 261 μg/mL).[51] In the meantime, by querying the China Pesticide Registration Database (http://www.chinapesticide.org.cn/hysj/index.jhtml), we found that the only two plant fungicides registered against *R. solani* within the validity period, namely, oligosaccharins and cnidiadin. The antifungal activity of cnidiadin (EC_{50}, 37.71 μg/mL) and oligosaccharins (EC_{50}, 34.56 μg/mL) against *R. solani* was better than that of the *M. cortex* petroleum ether extract.[50,51] However, we have isolated the pure natural product eugenol from the medicinal plant *Syzygium aromaticum*, and the EC_{50} value of eugenol against *R. solani* was 52.77 μg/mL, which showed excellent activity of inhibiting *R. solani*.[36] Therefore, we believe that it is possible to isolate pure compounds with higher antifungal activity against *R. solani* from the petroleum ether extract of *M. cortex*. Therefore, it is worth further studying to change the situation that there are few plant fungicides against *R. solani*.[52,53] Moreover, although the petroleum ether extract had excellent antifungal activity, its antifungal mode of action was not certain, which would limit its further development and utilization.

Studies have demonstrated that *M. cortex* has antifungal activity and its active ingredients may include natural products such as paeonol and quercetin[27,54] and the cell membrane may

https://doi.org/10.1021/acsomega.2c06150
ACS Omega 2022, 7, 47048−47055

be their target.[55,56] Therefore, we tested the extracellular electrical conductivity and OD_{260} of *R. solani* treated with the petroleum ether extract. The results showed that the extracellular electrical conductivity and the OD_{260} content of the mycelium in the treatment group were increased, indicating that the integrity of the cell membrane was injury, which caused leakage of intracellular substances to the outside of the cell. Moreover, the imbalance of electrolytes inside and outside the cell resulted in the inhibition of mycelial growth. It has been shown that intact cell membranes were critical for controlling the ingress and egress of substances and disruption often resulted in the leakage of small molecules (Na^+, K^+), intracellular proteins, and nucleic acids, out of the cell, resulting in loss of cellular function.[57,58] Our study is in agreement with the mechanism of action of the dichloromethane extract from *Streptomyces libani* and natural products eugenol and citral on the cell membranes.[59,60]

Ergosterol is the main component of the cell membrane, which plays an important role in the integrity of the cell membrane, the activity of membrane-binding enzymes, the fluidity of membrane, and the transportation of cell material.[61] Ergosterol is a special kind of sterol in fungi; if the synthesis of ergosterol is blocked, the structure and function of the membrane will be seriously damaged and finally lead to cell death.[62] In this study, we found that the petroleum ether extract of *M. cortex* reduced the content of ergosterol, which may be one of the reasons for the cell membrane damage. The results were consistent with the effects of the essential oil and alcohol extract of *Curcuma longa* on the ergosterol against *Aspergillus flavus* and *Fusarium graminearum*.[63,64]

In addition, we also found that the petroleum ether extract caused the increase of MDA content. MDA is a key indicator of cell membrane lipid peroxidation.[65] Excessive MDA will change the structure and function of biological macromolecules on the cell membrane, such as lipids, proteins, and nucleic acids, resulting in cell metabolic disorders and even death.[66] Therefore, we inferred that the petroleum ether extract of *M. cortex* inhibited the activity of *R. solani* by inducing excessive accumulation of the MDA content, resulting in increased membrane permeability, which may be one of the antifungal mechanisms of the petroleum ether extract of *M. cortex*. This is consistent with the antifungal mechanism of the *Eriobotrya japonica* leaf extract and the Chinese herbal extract compound (CHEC) on MDA.[67,68] The destruction of the cell membrane was also confirmed through the phenomenon of the mycelium being transparent and ruined by light microscopy. In the meantime, according to the increased transparency and breaking of the mycelium, we speculate that the petroleum ether extract may also do harm to the cell wall. It has been reported that drugs that damage the cell membrane can target the cell wall at the same time.[69] For instance, *Aspergillus flavus* was treated with aqueous extracts of *Tulbaghia violacea* Harv.; the content of β-(1,3) glucan and chitin and the production of glucan synthase and chitin synthase were reduced, indicating that the cell wall was damaged.[70] However, citral can induce the increase in chitinase activity of *Magnaporthe grisea*, which also indicated that the cell wall was damaged.[37] Therefore, how the petroleum ether extract of *M. cortex* affects the composition of the cell wall, such as the content of β-(1,3) glucose, chitin and chitinase activity, and whether it causes their increase or decrease, is our future research content.

In summary, this is the first report of the *M. cortex* extract exhibiting excellent antifungal activity against *R. solani*, and the active substance was mainly in the petroleum ether extract. Preliminary experimental exploration showed that the cell membrane may be the site of the action of the petroleum ether extract, thereby inhibiting the growth of the mycelium. In future research work, other antifungal targets and mechanisms of the petroleum ether extract of *M. cortex* are what we continue to explore. There is also the isolation and identification of monomeric active ingredients from the petroleum ether extract of *M. cortex*, to clarify the antifungal activity and mechanism of a single active ingredient, as well as the synergistic antifungal activity and mechanism of multiple active ingredients. This research will provide theoretical support for the development and utilization of *M. cortex* in phytosanitary fungicide for *R. solani*.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by the Education Quality Improvement Project of Qiannan Normal University for Nationalities (2021xjg018), the High-level Talent Research Project of Qiannan Normal University for Nationalities (2021qnsyrc07), the Science and Technology Project of Qiannan Science and Technology Bureau (2022qnkh09), the Innovation and Entrepreneurship Training Program for College Students of Guizhou Province (S202210670020, S202210670028), and the National Natural Science Foundation of China (31960605).

https://doi.org/10.1021/acsomega.2c06150
ACS Omega 2022, 7, 47048−47055

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