Int.J.Curr.Microbiol.App.Sci (2021) *10*(04): 180-188

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 04 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1004.017

Inhibition Effect of *Piper betle L.*, *Piper porphyrophyllum* N.E. Br. and *Piper aduncum* L. Leaf Extract on the Mycelial Growth of *Colletotrichum gloeosporioides* (Penz.) from a Rubber Tree

Fadli[1], Nasril Nasir[2], Abdi Dharma[1], Syafrizayanti[1] and Mai Efdi[1*]

[1]Department of Chemistry, [2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, KampusLimau Manis, Padang-25163, Indonesia

**Corresponding author*

Keywords

Antifungal, Piper betle, Piper porphyrophyllum, Piper aduncum, Colletotrichum gloeosporioides

Article Info

Accepted:
12 March 2021
Available Online:
10 April 2021

A B S T R A C T

Anthracnose leaf disease in rubber trees, caused by the attack of *Colletotrichum gloeosporioides*, have led to the serious decline in natural rubber production. Continuous usage of synthetic fungicide leave the toxic residues that harm the surrounding animals and plants and are not environmentally friendly. It needs a better solution with organic products that are safer, more effective and biodegradable. *Piper* species could be considered as a source of new natural products with potential antifungal activities. Thus, the research aimed to investigate the effect of leaf extracts from *Piper betle*, *P.porphyrophyllum* and *P.aduncum*in inhibiting the growth of *C. gloeosporioides* from rubber trees *in vitro*. Of the three *Piper* species, the crude methanolic extract of *P. porphyrophyllum* leaves shows the highest growth inhibition percentage (83.62%), followed by *P. aduncum* L. (79,38 %), *P. betle* (76.39%). In further examination on the leaf extract of *Piper* in various solvents, we find that the n-hexane extract of *P.aduncum*has the best performance in inhibiting the growth of *C. gloeosporioides* (GIP of 92.72%); it also beats the performance of Benomyl, a commercial fungicide, at the same concentration. The 50% percentage of growth inhibition (MIC_{50}) of this n-hexane extract towards *C. gloeosporioides* is reached at a very low concentration, 32 mg/L, while the MIC_{90} is reached at concentration of 799 mg/mL. The results suggest that the n-hexane leaf extract of *P. aduncum* is a potential candidate for anti-Colletotrichum agent.

Introduction

Rubber tree (*Hevea brasiliensis* Müll. Arg.)is the only major source for natural rubber production that is spread throughout the tropical regions of the world(Liu *et al.*, 2017). Natural rubber is a latex polymer with high elasticity, flexibility and resilience that plays an important role in the world economy and has not been completely replaced by synthetic rubber. *Colletotrichum gloeosporioides* is one of the deadly pathogens of the rubber tree that

causes a serious decline in natural rubber production (Wang *et al.,* 2018). *C. gloeosporioides* infects most of the rubber tree organs, mainly causing anthracnose thinning in rubber leaves. Circular spots on old or young leaves can be identified as a common symptom of this fungal disease. This pathogen also affects young branches with premature leaves that cause blackening or brown oval spots on the end of the new stems (Hunupolagama *et al.,* 2017).

The production of natural rubber can be increased if anthracnose disease is treated with a suitable synthetic fungicide. Synthetic fungicides such as Tridemorph, Benomyl and Bayfidan are quite effective in controlling rubber plant pathogenic fungi (Ogbebor *et al.,* 2015a). However, continuous usage of synthetic fungicide is very expensive for local farmers and the toxic residues harm the surrounding animals and plants and are not environmentally friendly (Aktar *et al.,* 2009; Kaewchai & Soytong, 2010). The recent increasing trend of positive consumer demand for organic products has created an encouragement for a search for fungicides that are safer, more effective and biodegradable.

Many researchers are interested in bio-fungicides with the use of plant extracts to control plant diseases because plant extracts are easily biodegradable, have very little residue in the soil and are harmless to animals and humans (Zarins *et al.,* 2009; Rashid *et al.,* 2014). Piperaceae is large and widely diverse plants distributed in a tropical forest in Asia. It consists of more than 3000 species where the *Piper* genus has the most popular and large biodiversity consists of more than 2000 species (Perigo *et al.,* 2016). *Piper* is a large and highly diverse genus of plants with medicinal use. It is widely distributed in tropical forests occupying distinct environments. *Piper* species have been extensively investigated as a source of new natural products with potential bioactivities such as antifungals. The search for new antifungal resources will contribute to establish a model for further investments in this field to preserve and study the flora especially the *Piper* species.

The control of *C. gloeosporioides* with extracts from Piperaceae has been carried out so far by Silva *et al.,* (2014)who reported that the antifungal water extract of *Piper hispidinervum* could inhibit *C. gloeosporioides* coffee plant isolates by 46.37% at a concentration of 20%. Bussaman *et al.,* (2012)also reported that the crude extract of Methanol from *P. sarmentosum* leaves can actively (100%) inhibit the growth of fungal mycelium isolates *C. gloeosporioides* from mango plants at a concentration of 0.25% using the food poisoned technique method. Radwan *et al.,* (2014) reported that *P. nigrum* showed antifungal activity against *C. gloeosporioides* isolates from strawberry plants with an inhibition zone of 5.5 mm at 80 µg / spot. However, no antifungal information related to extract from leaves of *Piper betle, Piper porphyrophyllum* and *Piper aduncum* against *Colletotrichum goeosporioides* was found in the literature during the preparation of this manuscript. Therefore, the authors want to assesses the antifungal activity of the extract of the leaves of that three *Piper* species against *C. gloeosporioides* which can later be used as a reference for isolating sources of potential active compounds.

Materials and Methods

Plant material

The plants were grown in the experimental field of the Biological Education and Research Forest (HPPB), Andalas University, Padang, Indonesia. Three of *Piper* species were collected from October- December 2019by

Fadli, Muhammad Ikhsan, and Firham Yasra. Voucher specimens were deposited and identified by the plant taxonomists (accession numbers ANDA00021318 [*Piper betle*], ANDA00021321 [*P. porphyrophyllum*], and ANDA00021317 [*P. aduncum*]; Suppl. Data S1). at the Herbarium of Andalas University (ANDA) and Herbarium Bogoriense (BO). Leaves of each plant were dried and powdered.

Fungal strain and cultivation

Colletotrichum gloeosporioides isolate was obtained from infected leaves of rubber trees (*Hevea brasiliensis*) in Sembawa Research Center, Indonesian Rubber Research Institute, South Sumatra. Furthermore, the fungal isolates were cultured in PDA medium (20 g agar, 20 g glucose, 200 g potato and 1000 mL distilled water, autoclaved at 121° C for 30 minutes) slanted under dark conditions at 5 ° C until further use.

Preparation of Plant Extract

To make a crude leaf extract, the dried and powdered leaves were soaked with analytical grade methanol in a dark bottle kept at room temperature for 72 hours (maceration was repeated six times). The methanol extract was then filtered with Whatman filter paper No. 1 and concentrated at 40°C under low pressure using a rotary evaporator (IKA-RV 10 with refrigeration unit). Crude methanol extract from three *Piper* species was then re-extracted with n-hexane, ethyl acetate, and hydromethanol (residue), and concentrated again (Rodrigues *et al.*, 2012).

Antifungal examination

The antifungal activity was carried out according to the food poisoned technique by Guerrini *et al.*, (2009) with modification, fungal cultures were grown on potato dextrose agar (PDA, Merck). The extract of *Piper betle*, *Piper porphyrophyllum* and *Piper aduncum* leaves were dissolved in DMSO and aseptically added to sterile media at 45 ° C. Here, the methanolic crude extracts from three *Piper* spp. were tested against *C. gloeosporioides*at a concentration of 2500 mg/L, then the re-extracted methanolic crude extracts (n-hexane, ethyl acetate and hydromethanol) also tested further against *C. gloeosporioides* of 1000 mg/L. The DMSO concentration in the final solution was adjusted to 1%. Control with the amount of DMSO without extract equivalent and positive control with commercial fungicide Benomyl 1000 mg/L.

The culture was obtained by removing the mycelium disk (6 mm diameter) from the pure parent culture in a stationary phase at 26 ± 1 ° C until a logarithmic growth phase was achieved. Subsequently the culture in the final logarithmic phase was transferred to a Petri dish with the medium containing the extract diluted to the final concentrations mentioned above. Fungal growth was evaluated daily by measuring the culture diameter for 5 days of treatment. There were three replications for each treatment. Treatment and control mycelium growth in petri dishes was calculated based on the mycelium diameter using a vernier caliper. The percentage of inhibition is calculated based on (Liu *et al.*, 2017) using the formula: *Growth Inhibition Percentage* (GIP, %) = (colony diameter of control − colony diameter of treatment) / (colony diameter of control − mycelial disk diameter) × 100, where mycelial disk diameter is 6 mm (see above).

MIC_{50} and MIC_{90} Determination

To calculate the minimum inhibitory concentration (MIC), a series of 2-folds extract concentration (31,25, 62,5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L) from

the best extract of the latter examination was tested against *C. gloeosporioides*using the same modified method of (Bussaman *et al.,* 2012). For all treatments, media with DMSO but with no leaf extract was used for negative control and media with DMSO and a commercial fungicide Benomyl from Benlate® (DUPON) was used for positive control at 1000 mg/L. The MIC_{90} and MIC_{50} were calculated based on the interpolation of the relationship between the GIP and concentration values from the best extract. The MIC_{90} and MIC_{50}refer to the concentration cut points where the percentage of growth inhibition is 90% and 50%, respectively (España *et al.,* 2017). MIC_{90} is considered as the best parameter for antifungal activity, whereas MIC_{50} is considered as an effective and significant concentration (Griffin *et al.,* 2000; España *et al.,* 2017).

Statistical Analysis

Descriptive statistical analysis was performed using SPSS (version 26.0) for Windows (SPSS, Inc., Chicago, IL). The GIP data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test.

Results and Discussion

The results of the in vitro antifungal activity of Piper leaves extract in HPPB against the fungus *C. gloeosporioides* are shown in Figure 1. Three types of Piper plants showed different percentages of growth inhibition against the tested fungi. Interestingly, the crude methanol extract of the three Piper leaves showed more effective inhibitory activity than the positive control Benomyl against the growth of *C. gloeosporioides* mycelium with the inhibition percentage of *P. porphyrophyllum* extract (83.62%), followed by *P. aduncum* L. (79,38 %), *P. betle* (76.39%). Not many research have studied the potential of *Piper* plants against *C. gloeosporioides* isolate from rubber plant. The control of *C. gloeosporioides* with extracts from Piperaceae has been carried out so far by Silva *et al.,* (2014)who reported that the antifungal water extract of *Piper hispidinervum* could inhibit *C. gloeosporioides* coffee plant isolates by 46.37% at a concentration of 20%. Bussaman *et al.,* (2012)also reported that the crude extract of Methanol from *P. sarmentosum* leaves can actively (100%) inhibit the growth of fungal mycelium isolates *C. gloeosporioides* from mango plants at a concentration of 0.25% using the food poisoned technique. Ogbebor *et al.,* (2010)reported that the most efficient extracts in controlling the *C. gloeosporioides* were *Allium sativum* and *Cymbopogon citratus* with complete inhibition of the growth of *C. gloeosporioides* mycelium at 10, 25, 50 and 100%.

The potential of *P. porphyrophyllum* extract is promising to be used as an antifungal source. However, other pipers such as *Piper aduncum* and *P. betle* also showed strong antifungal activity against the rubber plant pathogenic fungus *Colletotrichum gloeosporioides*.

Therefore, crude methanol extracts of *P. aduncum*, *P. porphyrophyllum* and *P. betle* leaves were fractionated with solvent to produce n-hexane, ethyl acetate and Hydromethanol extracts.

The GIP of *P. aduncum*n-hexane leaf extract against *C. gloeosporioides* was very high and significant among other extract with a GIP value of 92.72%. In addition, this extract had a higher GIP value than Benomyl positive control at the same concentration against *C. gloeosporioides*. So far no one has reported the GIP value of *P. aduncum* leaf hexane extract against the fungus *C. gloeosporioides*, a rubber plant pathogen.

Fig.1 GIP of *Colletotrichum gloeosporioides* by crude methanolic leaf extract of *Piper* at a concentrationof 2500 mg / L and Benomyl 1000 mg / L as a control. The data shown represent the mean of three replicate trials of the mycelium diameter of the treatment on the same day. The lower case letters above the error bars represent the mean groups, different letters indicate that the groups are significantly different from each other ($p < 0.05$)

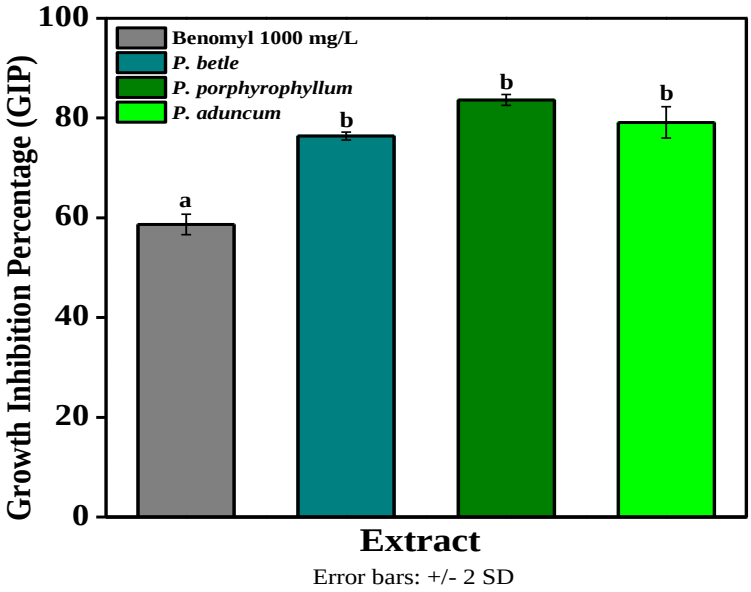


Fig.2 GIP (%) of 1000 mg / L extract of hexane, ethyl acetate, and hydromethanol from *Piper betle*, *P. porphyrophyllum* and *P. aduncum* against *Colletotrichum gloeosporioides*. Annotation as in Fig. 1.

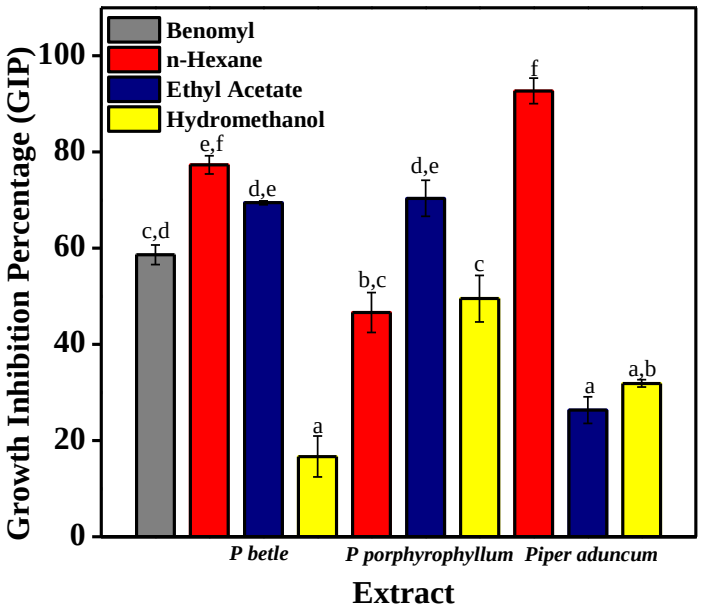
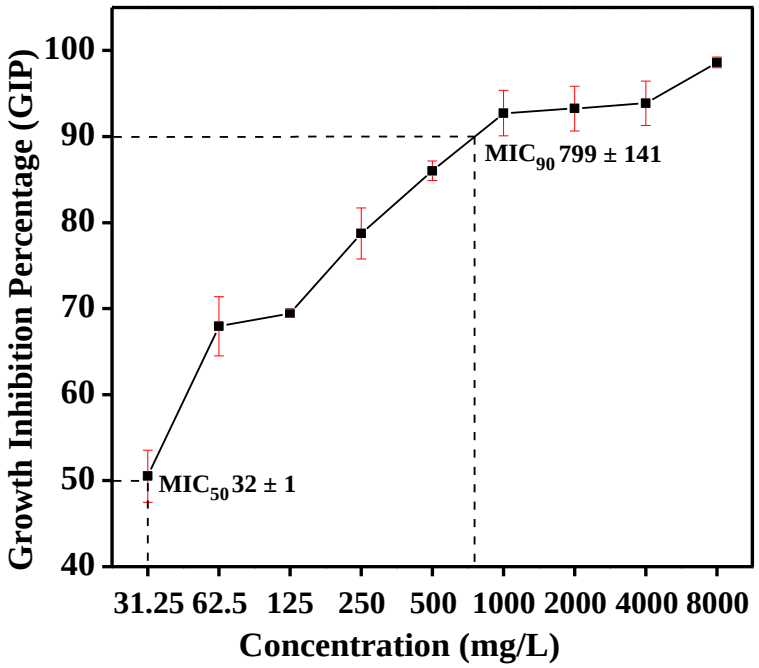


Fig.3 MIC_{90} and MIC_{50} values of n-hexane extract of *P. aduncum* leaves against *C. gloeosporioides*, with 2-folds dilution in range of 31.25-8000 mg/L concentration, as shown as concentration values above each bar. Standard deviation is shown as error line on each point.



The most potent extract of *Piper* species is the *P. aduncum* hexane extract, which significantly inhibits fungal growth at a concentration of 1000 mg/L with a percentage value of inhibition greater than the extracts of other *Piper* species tested. Therefore, n-hexane extract of *Piper aduncum* was selected to be further tested to determine the Minimum Inhibitory Concentration 90% and 50% (MIC_{90} and MIC_{50}).

The minimum inhibition concentrations of 90 and 50 (MIC_{90} and MIC_{50}) were calculated based on interpolation from the GIP (growth inhibition percentage) associated data with the concentration values, referring to the concentration cutoff point where the inhibition was 90% and 50%, respectively (España *et al.*, 2017).The growth inhibition percentage increases as increasing concentration used. N-hexane extract of *P. aduncum* was very good at inhibiting the growth of *Colletotrichum gloeosporioides* with a low MIC_{90} concentration value (799 mg/L).

The results of this study indicate that the *P. aduncum*n-hexane leaf extract has good potential in inhibiting the fungus *C. gloeosporioides* compared to the results of other studies by España *et al.*, (2017)who reported that the ethanol extract of the *Eucalyptus* wood industrial by-product, the leaves of *Eucalyptus* showed strong inhibition against *C. gloeosporioides*.

The ethanolic extract of *Eucalyptus camaldulensis* showed a strong antifungal MIC_{90} value (98% GIP) at high concentrations (5000 mg/L). This means that the hexane extract of *P. aduncum* is better at inhibiting the growth of *C. gloeosporioides* with a lower MIC_{90}concentration at 799 mg/L which is much lower than the ethanol extract of *E. camaldulensis*. In another study, the strongest

fraction that inhibited the growth of *C. gloeosporioides* was ethanolic extract of *Silene armeria* L. with an MIC value of 1000 mg / L, between 1500 and 5000 mg / L for *Avicennias chaueriana* and higher than 10000 mg / L for *Carica papaya* L. (Bajpai *et al.*, 2008; Chávez-Quintal *et al.*, 2011; Fardin & Young, 2015).

España *et al.*, (2017)also reported that the ethanolic extract of *Eucalyptus globulus* showed significant antifungal activity of MIC_{50} (50% GIP) at low concentration (500 mg / L) against *C. gloeosporioides*. Interestingly, at lower concentrations, the n-hexane extract of *P. aduncum* in this study showed a growth inhibition half value (MIC_{50}) only at a concentration of 32 mg / L against *C. gloeosporioides* which was stronger than that of España *et al.*, (2017).

This research shows that the possibility of a chemical component that is very active in inhibiting the *C. gloeosporioides*, a rubber plant fungal pathogenic, is present in *P. aduncum* leaves extracted by diffusion in n-hexane. So that the maceration method using n-hexane is a suitable way to obtain bioactive extracts from this plant.

In addition, it is also necessary to conduct a profile analysis of the compounds contained in *P. aduncum*n-hexane extract to determine which compounds have the potential as biofungicides against pathogenic fungi of rubber plants so that they can be used as a reference for further research on the isolation of active compounds from these plants.

Acknowledgments

The author thanks to the Ministry of Research, Technology and Higher Education (Ristekdikti) Republic of Indonesia that was supported this research through PMDSU program (contract number 163/SP2H/ LT/DRPM/2019).

References

Aktar, M. W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2, 1–12. https://doi.org/10.2478/v10102-009-0001-7

Bajpai, V. K., Shukla, S., Kang, S. C., 2008. Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2008.04.060

Bussaman, P., Namsena, P., Rattanasena, P., Chandrapatya, A., 2012. Effect of crude leaf extracts on *Colletotrichum gloeosporioides* (Penz.) Sacc. Psyche (New York) 2012. https://doi.org/10.1155/2012/309046

Chávez-Quintal, P., González-Flores, T., Rodríguez-Buenfil, I., Gallegos-Tintoré, S., 2011. Antifungal Activity in Ethanolic Extracts of *Carica papaya* L. cv. Maradol Leaves and Seeds. Indian J. Microbiol. https://doi.org/10.1007/s12088-011-0086-5

España, M. D., Arboleda, J. W., Ribeiro, J. A., Abdelnur, P. V., Guzman, J. D., 2017a. Eucalyptus leaf byproduct inhibits the anthracnose-causing fungus *Colletotrichum gloeosporioides*. Ind. Crops Prod. 108, 793–797. https://doi.org/10.1016/j.indcrop.2017.08.002

España, M. D., Arboleda, J. W., Ribeiro, J. A., Abdelnur, P. V., Guzman, J.D., 2017b. Eucalyptus leaf by product inhibits the anthracnose-causing fungus *Colletotrichum gloeosporioides*. Ind. Crops Prod. 108, 793–797.

https://doi.org/10.1016/j.indcrop.2017.08.002

Fardin, K. M., Young, M. C. M., 2015. Antifungal potential of *Avicennia schaueriana* Stapf & Leech. (Acanthaceae) against *Cladosporium* and *Colletotrichum* species. Lett. Appl. Microbiol. https://doi.org/10.1111/lam.12423

Griffin, S. G., Markham, J. L., Leach, D. N., 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Essent. Oil Res. 12, 249–255. https://doi.org/10.1080/10412905.2000.9699509.

Guerrini, A., Sacchetti, G., Rossi, D., Paganetto, G., Muzzoli, M., Andreotti, E., Tognolini, M., Maldonado, M. E., Bruni, R., 2009. Bioactivities of *Piper aduncum* L. and Piper obliquum Ruiz & Pavon (*Piperaceae*) essential oils from Eastern Ecuador. Environ. Toxicol. Pharmacol. 27, 39–48. https://doi.org/10.1016/j.etap.2008.08.002

Hunupolagama, D. M., Chandrasekharan, N. V., Wijesundera, W. S. S., Kathriarachchi, H. S., Fernando, T. H. P. S., Wijesundera, R. L. C., 2017. Unveiling Members of Colletotrichum acutatum Species Complex Causing Colletotrichum Leaf Disease of Hevea brasiliensis in Sri Lanka. Curr. Microbiol. 74, 747–756. https://doi.org/10.1007/s00284-017-1238-6

Kaewchai, S., Soytong, K., 2010. Application of biofungicides against Rigidoporus microporus causing white root disease of rubber trees. J. Agric. Technol. 6, 349–363.

Liu, X., Ouyang, C., Wang, Q., Li, Y., Yan, D., Yang, D., Fang, W., Cao, A., Guo, M., 2017. Effects of oil extracts of Eupatorium adenophorum on Phytophthora capsici and other plant pathogenic fungi in vitro. Pestic. Biochem. Physiol. 140, 90–96. https://doi.org/10.1016/j.pestbp.2017.06.012

Liu, Z., Wu, M., Ke, Z., Liu, W., Li, X., 2017. Functional analysis of a regulator of G-protein signaling CgRGS1 in the rubber tree anthracnose fungus *Colletotrichum gloeosporioides*. Arch. Microbiol. 0, 0. https://doi.org/10.1007/s00203-017-1455-1

Ogbebor, N., Adekunle, A., Enobakhare, D., 2010. Inhibition of *Colletotrichum gloeosporioides* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis* Muell. Arg.) leaf spot using plant extracts. African J. Biotechnol.

Ogbebor, N. O., Adekunle, A. T., Eghafona, N.., Ikponmwosa, A., 2015. In vitro and in vivo synthetic fungicides control of Rigidoporus microporus on Para rubber in Nigeria. J. Crop Prot 4, 477–485.

Perigo, C. V., Torres, R. B., Bernacci, L. C., Guimarães, E. F., Haber, L. L., Facanali, R., Vieira, M. A. R., Quecini, V., Marques, M. O. M., 2016. The chemical composition and antibacterial activity of eleven Piper species from distinct rainforest areas in Southeastern Brazil. Ind. Crops Prod. 94, 528–539. https://doi.org/10.1016/j.indcrop.2016.09.028

Radwan, M. M., Tabanca, N., Wedge, D. E., Tarawneh, A. H., Cutler, S. J., 2014. Antifungal compounds from turmeric and nutmeg with activity against plant pathogens. Fitoterapia 99, 341–346. https://doi.org/10.1016/j.fitote.2014.08.021

Rashid, M. M., Kabir, M. H., Hossain, M. M., Bhuiyan, M. R., Iqbal Khan, M. A., 2014. Eco-Friendly Management of Chilli Anthracnose (*Colletotrichum capsici*). Int. J. Plant Pathol. https://doi.org/10.3923/ijpp.2015.1.11

Rodrigues, E. R., Nogueira, N. G. P., Zocolo, G. J., Leite, F. S., Januario, A. H., Fusco-Almeida, A. M., Fachin, A. L., de Marchi, M. R. R., dos Santos, A. G., Pietro, R. C. L. R., 2012. Pothomorphe umbellata: Antifungal activity against strains of Trichophyton rubrum. J. Mycol. Med. 22, 265–269. https://doi.org/10.1016/j.mycmed.2012.05.005

Silva, J., Souza, P. E., Monteiro, F. P., 2014. Antifungal activity using medicinal plant extracts against pathogens of coffee tree. Rev. Bras. Pl. Med., Campinas 16, 539–544.

Wang, W., An, B., Feng, L., He, C., Luo, H., 2018. A *Colletotrichum gloeosporioides* Cerato-platanin protein, CgCP1, contributes to the conidiation and plays roles in the interaction with rubber tree. Can. J. Microbiol. 1–33.

Zarins, I., Daugavietis, M., Halimona, J., 2009. Biological activity of plant extracts and their application as ecologically harmless biopesticide. Sci. Work. Lith. Inst. Hortic. Lith. univeristy agricuture 28, 269–280.

How to cite this article:

Fadli, Nasril Nasir, Abdi Dharma, Syafrizayanti and Mai Efdi. 2021. Inhibition Effect of *Piper betle L., Piper porphyrophyllum* N.E. Br. and *Piper aduncum* L. Leaf Extract on the Mycelial Growth of *Colletotrichum gloeosporioides* (Penz.) from a Rubber Tree. *Int.J.Curr.Microbiol.App.Sci.* 10(04): 180-188. doi: https://doi.org/10.20546/ijcmas.2021.1004.017