Journal of Applied and Natural Science
16(1), 420 - 426 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Active compounds of *Michelia champaca* bark extract against *Curvularia verruculosa* fungi causing leaf spot disease in rice (*Oryza sativa* L.)

I Gusti Agung Gede Bawa*
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Sri Rahayu Santi
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Wiwik Susanah Rita
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Olan Suryanadi
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Gek Indyan
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia

*Corresponding author. E-mail: gede_bawa@unud.ac.id

Article Info
https://doi.org/10.31018/jans.v16i1.5406
Received: January 8, 2024
Revised: March 1, 2024
Accepted: March 10, 2024

How to Cite
Bawa, I.G.A.G. *et al.* (2024). Active compounds of *Michelia champaca* bark extract against *Curvularia verruculosa* fungi causing leaf spot disease in rice (*Oryza sativa* L.). Journal of Applied and Natural Science, 16(1), 420 - 426. https://doi.org/10.31018/jans.v16i1.5406

Abstract
Curvularia verruculosa fungal causes leaf spot disease in rice plants. The bark extract of *Michelia champaca* could inhibit the growth of the fungi. The present research aimed to know the active compound responsible for antifungal activity. Extraction was done using the Maceration method, antifungal activity was measured using the Diffusion well method, and identification of active compounds was carried out using Gas chromatography-mass spectroscopy (GC-MS). The methanol extract obtained had a yield of 18.7%. It showed strong activity against *C. verruculosa* with an inhibition zone until 30.01 mm. The fractionation results showed that n-hexane extract (HE) was the strongest inhibiting the growth of C. verruculosa (32.45 mm), followed by chloroform extract (CE) (29.20 mm), while n-butanol extract (BE) was not active. Separating active compounds from HE extract was made using Column chromatography (CC) method with silica gel as the stationary phase and the mixture of n-hexane-acetone (3:1) as the mobile phase. This separation resulted in 5 combined fractions; HE3 and HE5 extracts showed very strong activity against *C. verruculosa*, with a diameter of the inhibition zone of 26.73 and 33.46 mm, respectively; HE2 extract showed strong activity with a diameter of the inhibition zone of 15.21 mm, while HE1 and HE4 extracts did not show activity. Identification using GC-MS, especially the HE3 extract, revealed that the extract contained two compounds: tributyl acetyl citrate and terephthalic acid, dodecyl-2-ethylhexyl ester. The result indicated that the bark extract of *M. champaca* had the potential to be a botanical fungicide.

Keywords: Antifungal activity, *Curvularia verruculosa*, *Michelia champaca*, Terephthalic acid, dodecyl-2-ethylhexyl ester, Tributyl acetyl citrate

INTRODUCTION

The fungus *Curvularia verruculosa* causes leaf spot disease in Ciherang rice plants in Ayunan Village, Bali (Bawa, 2019). Currently, the control of this disease still uses various kinds of synthetic fungicides after the appearance of symptoms, but they do not give satisfactory results. The uncontrolled use of synthetic fungicides will harm farmers and the environment (West *et al*., 2003; Yoon *et al*., 2013). The development of botanical fungicides is an alternative because they contain various bioactive compounds with residues that are easily degraded in nature and are not phytotoxic (Kagale *et al*., 2005; Omezzine *et al*., 2011). One of the botanical

fungicides developed is *Michelia alba* bark extract (Bawa and Bogoriani, 2021). Besides that, the extract of *Michelia champaca* bark also showed a very strong inhibitory effect on the growth of *C. verruculosa* fungi with an inhibition zone of 30.01 mm, so it has the potential to be developed into a botanical fungicide for *C. verruculosa* fungi (Bawa, 2021). Several uses of this plant as a botanical fungicide have been reported. Mangang and Chhetry (2012) found that *M. champaca* plant extract at a concentration of 5% was able to inhibit the growth of the mycelia of *Rhizoctonia solani* fungi which causes root rot disease in chickpeas (*Phaseolus vulgaris*) with an inhibition percentage of 49.54%, while Kumar *et al.* (2011) reported that the crude extract of *M. champaca* showed high antifungal activity against Candida albicans fungi. Pawar (2015) reported that the root extract of *M. champaca* inhibited the growth of pathogenic fungi carried by *Curvularia lunata* seeds with an inhibition zone of 15 mm.

The antifungal activity of *M. champaca* is probably due to the content of bioactive compounds, particularly its secondary metabolites. Huang *et al.* (2014) reported that *M. champaca* stems contain champacaine, (–)-anonaine, (–)-norushinsunine, (–)-ushinsunine, (–)-N-acetylanonaine, (–)-roemerine, (–)-asimilobine compounds. , (–)-anolobine, (–)-isocorydine, liriodenine, atherospermidine, O-methylmoschatoline, (+)-syringaresinol, N-trans-feruloyltyramine, 4-hydroxybenzaldehyde, vanillin, vanillic acid, syringic acid, 3,4-dimethoxybenzoic acid, coniferyl aldehyde, syringin, scopoletin, 4-acetonyl-3,5-dimethoxy-p-quinol, -sitostenone, and stigmasta-4,22-dien-3-one. Since *M. champaca* bark extract is responsible for inhibiting the growth of *C. verruculosa* fungi, the present study aimed to isolate and identify the active compounds in *C. verruculosa* that cause leaf spot disease in rice (*Oryza sativa* L.).

MATERIALS AND METHODS

Material

Michelia champaca bark was collected in Bongkasa Village, Badung, Bali and strain of *C. verruculosa* CI-MAP Og 22018 was isolated from rice leaves infected with leaf spot disease in Ayunan Village, Badung Bali and these fungi were deposited in Biopesticide Laboratory Udayana University.

Instrument

Gas chromatography-Mass spectroscopy GCMS-QP2010S Shimadzu Corporation, Japan 2017; with AGILENT DB-1 (CrossbondR 100% dimethylpolysiloxane) column 30 m long; ID: 0.25mm; film thickness 0.2 µm; carrier gas : Helium; ionizing strength of 70 eV was used.

Methodology extraction

The bark of *M. champaca* was collected from Jehem village, Bali, in June 2022 and brought to the Natural Product Chemistry Laboratory at Udayana University. They were cleaned in running tap water and air-dried for a day. The dried samples were cut into small pieces and dried again. Samples were blended to dry to a powder. 1 kg of dry sample powder was macerated with 2 L methanol for 24 hours 3 times. The filtrates obtained were combined and the solvent evaporated using a rotary vacuum evaporator to obtain crude methanol extracts.

Bioassay

A Petri dish with a diameter of 9 cm was filled with 1 ml of finely chopped *C. verruculossa* fungi culture suspension and dissolved in sterile water, then 10 ml of dilute Potato Dextrose Agar (PDA) medium (temperature around 45^{o}C) was shaken horizontally so that the fungi and PDA media were thoroughly mixed. After the media had solidified, two diffusion wells were made in each Petri dish. Each diffusion well was filled with 20 µl of *M. champaca* bark extract. This culture was placed in a dark place at room temperature. Observations were made by measuring the diameter of the inhibition zone formed (in mm) around the diffusion well.

Fractionation

Fractionation of the crude extract of *M. champaca* bark was carried out to separate the components of the compound based on their solubility. 30 g of crude extract of *M. champaca* bark dissolved in 250 ml of 70% ethanol solvent. This solution was evaporated by a rotary vacuum evaporator until all ethanol solvent evaporated. Water extract was fractionated successively with 3 x 100 ml of n-hexane, chloroform, and 1-butanol sol-

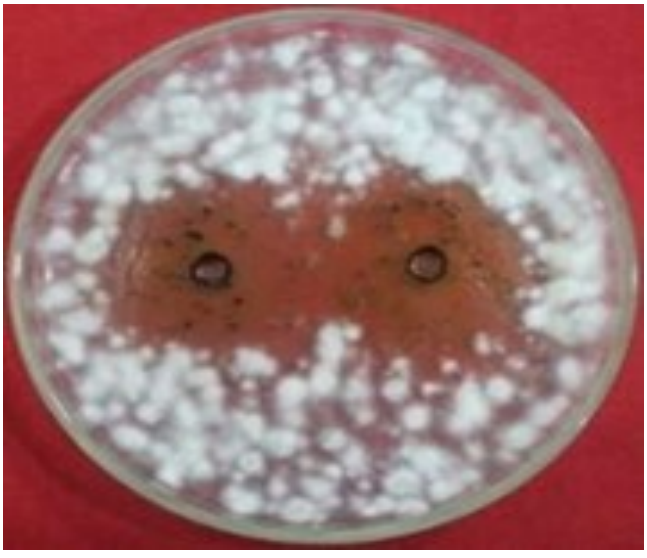

Fig.1. *Inhibition zone of crude extract of* Michelia champaca *bark to* C. verruculosa *fungal*

vents. The solvent from each fraction was evaporated using a rotary vacuum evaporator to obtain n-hexane (HE), chloroform (KE), 1-butanol (BE), and water extract (W). Each extract was tested for its antifungal activity *in vitro*.

Separation

Extracts showing antifungal activity against *C. verruculossa* were separated by Column chromatography. As much as 2 g of extract was put into the chromatography column of 59 cm long and 3.2 cm in diameter. The column was previously filled with 100 g of silica gel (Wako gel, particle size 75-150 µm) and solidified for 24 hours. The best eluent flowed column was obtained using thin-layer chromatography. The eluate was collected every 5 ml and tested by Thin-layer chromatography. Fractions showing the same stain pattern were combined. Each combined fraction was tested using the Well-diffusion method for its inhibitory activity against *C. verruculossa*.

Analysis of active compounds

Each active antifungal fraction was analyzed by Gas chromatography-mass spectroscopy (GCMS-QP2010S Shimadzu Corporation, Japan 2017).

RESULTS AND DISCUSSION

Extraction and fractionation

The extracted yield of *M. champaca* bark with methanol solvent was 17.8%. The crude extract of *M. champaca* bark at a concentration of 10% could strongly inhibit the growth of *C. verruculosa* fungi with an inhibition zone reaching 30.01 mm (Fig. 1). The partitioned yield of the crude extract with hexane, chloroform, and butanol solvents, successively, was 3.65 g of the hexane extract (HE); 15.32 g of a chloroform extract (CE), and 23.20 g of butanol extract, while the water fraction was not further processed. The antifungal activity of HE and CE extracts at a concentration of 1% can very strongly inhibit the growth of *C. verruculosa* with an inhibition zone of 32.45 and 29.20 mm respectively, while the butanol extract was not active. The antifungal activity of each extract against *C. verruculosa* is presented in Table 1 and Fig. 2.

Table 1. Inhibition zone of the fractionation yields of crude extract of *Michelia champaca* bark to *C. verruculosa*

Extracts	Inhibition Zone (mm)
Hexane Extract (HE)	32.45
Chloroform Extract (CE)	29.20
Butanol Extract (BE)	0.00

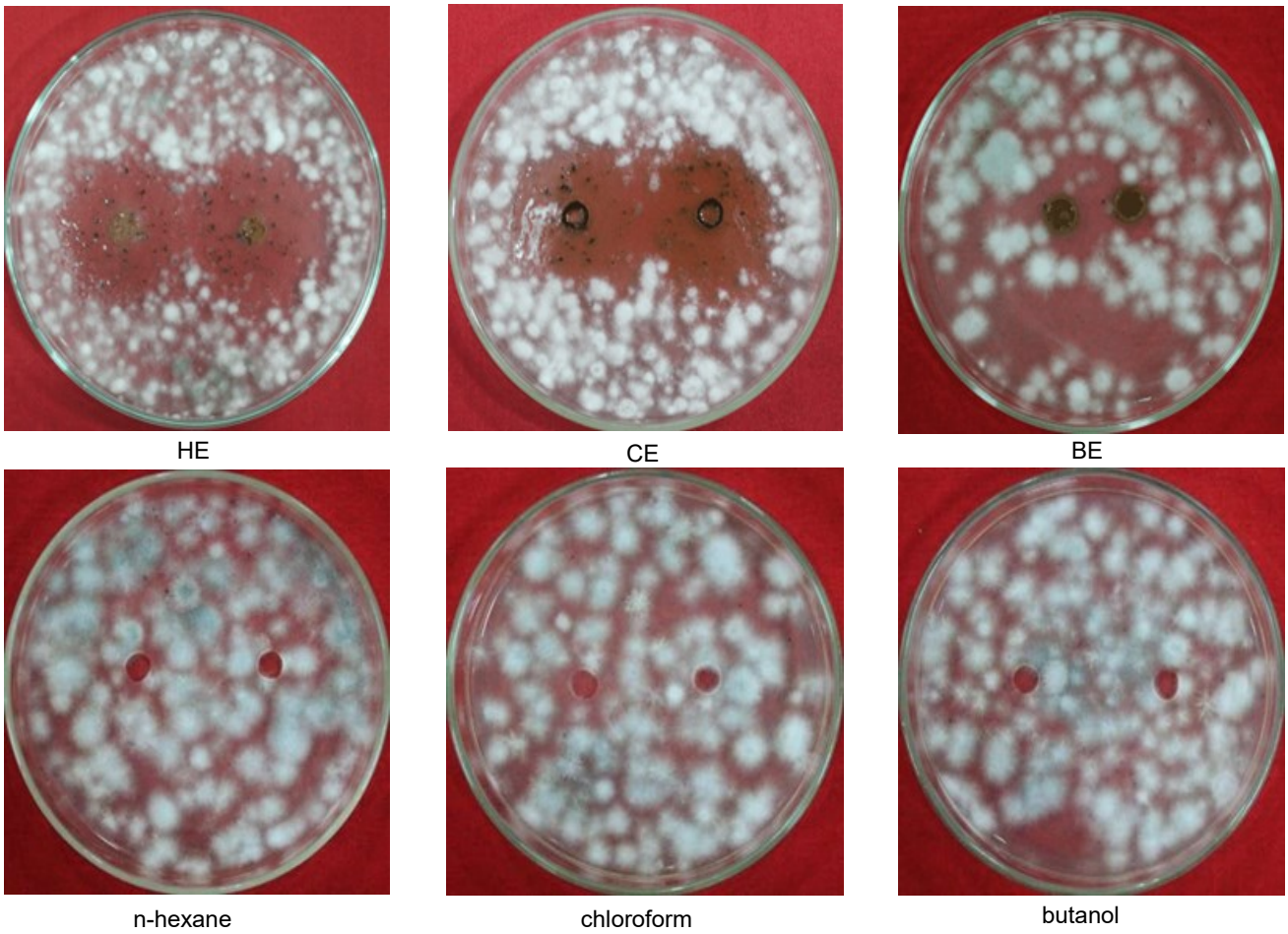

Fig. 2. *Inhibition zone of fractionation yields of crude extract of Michelia champaca bark and solvents to C. verruculosa fungi*

Table 2. Inhibition zone of separation yields of hexane extract (HE) of *Michelia champaca* bark to *C. verruculosa* fungi

Combined Fraction	Extracts	Inhibition Zone (mm)
1 - 26	HE1	0.00
27 - 39	HE2	15.21
40 - 63	HE3	26.73
64 -79	HE4	0.00
80 - 118	HE5	33.46

Separation of active compounds

Based on the antifungal activity, HE extracts showed the strongest antifungal activity. The separation of the extract by the Column chromatography with silica gel 60 as stationary phase and hexane : acetone (3:1) as mobile phase produced 5 fractions: HE1, HE2, HE3, HE4, and HE5. The antifungal activity of these extracts showed that HE3 and HE5 extracts could very strongly inhibit the growth of *C. verruculosa* fungal, HE2 extract could strongly inhibit it, while HE1 and HE4 extract were not active. The antifungal activity of separation yields of HE extract against *C. verruculosa* is mentioned in Table 2 and Fig. 3.

Identification of the HE3 extract by Gas Chromatography-Mass Spectroscopy (GCMS)

The GCMS of the HE3 extract is shown in Fig. 4, and the Mass spectroscopy spectrum of each peak is presented in Fig. 5 and 6. Fig. 4 shows that the HE3 ex-

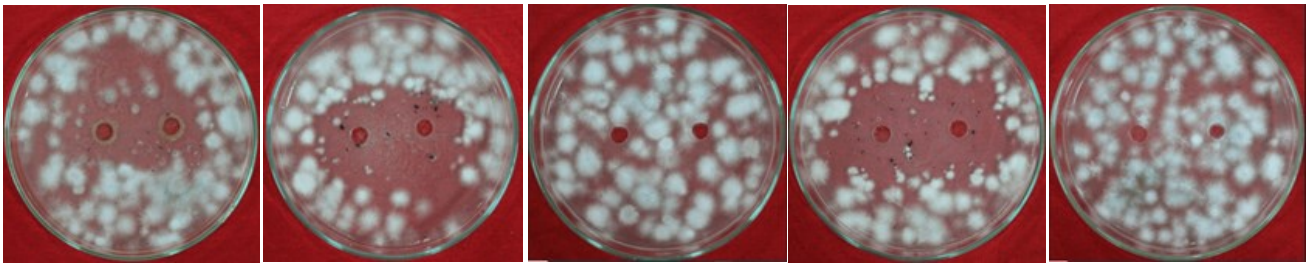

Fig. 3. *Inhibition zone of separation yields of HE extract of* Michelia champaca *bark to* C. verruculosa *fungi*

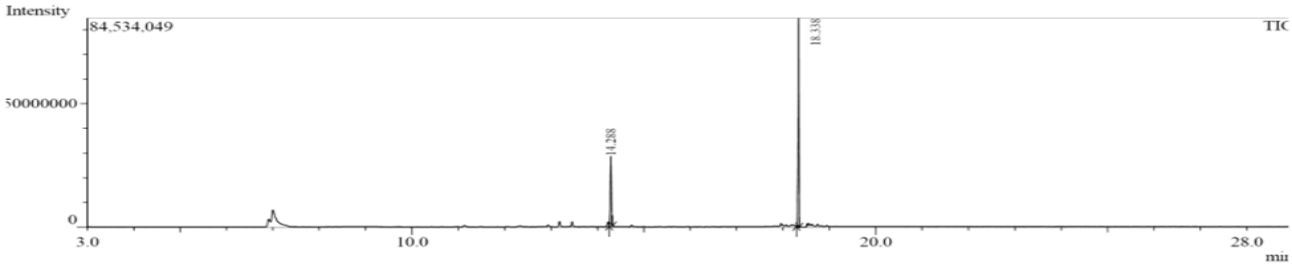

Fig. 4. *Chromatogram of HE3 fraction of Michelia champaca bark extract*

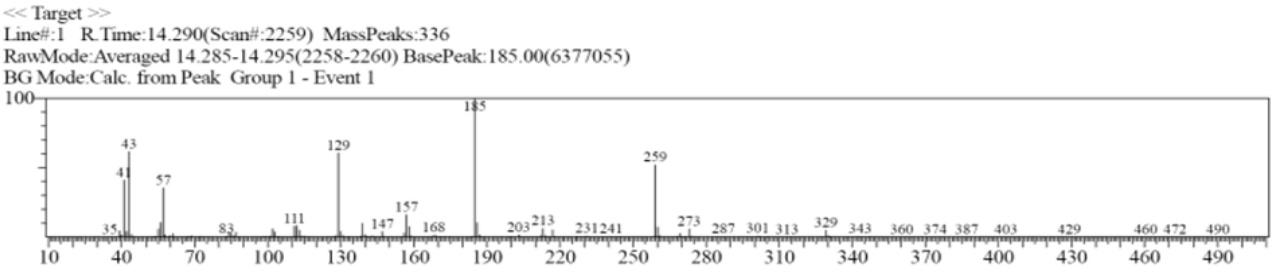

Fig. 5. *GC-MS spectrum of compound 1 in HE3 fraction of Michelia champaca bark extract*

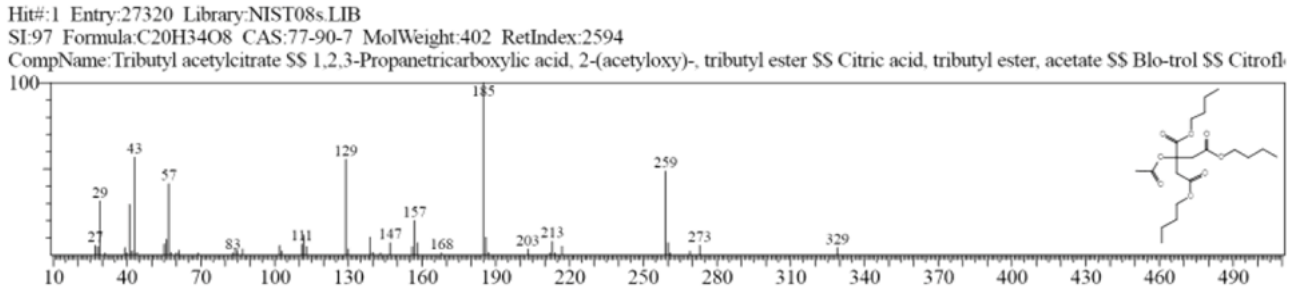

Fig. 6. *GC-MS spectrum of reference compound 1 in HE3 fraction of Michelia champaca bark extract*

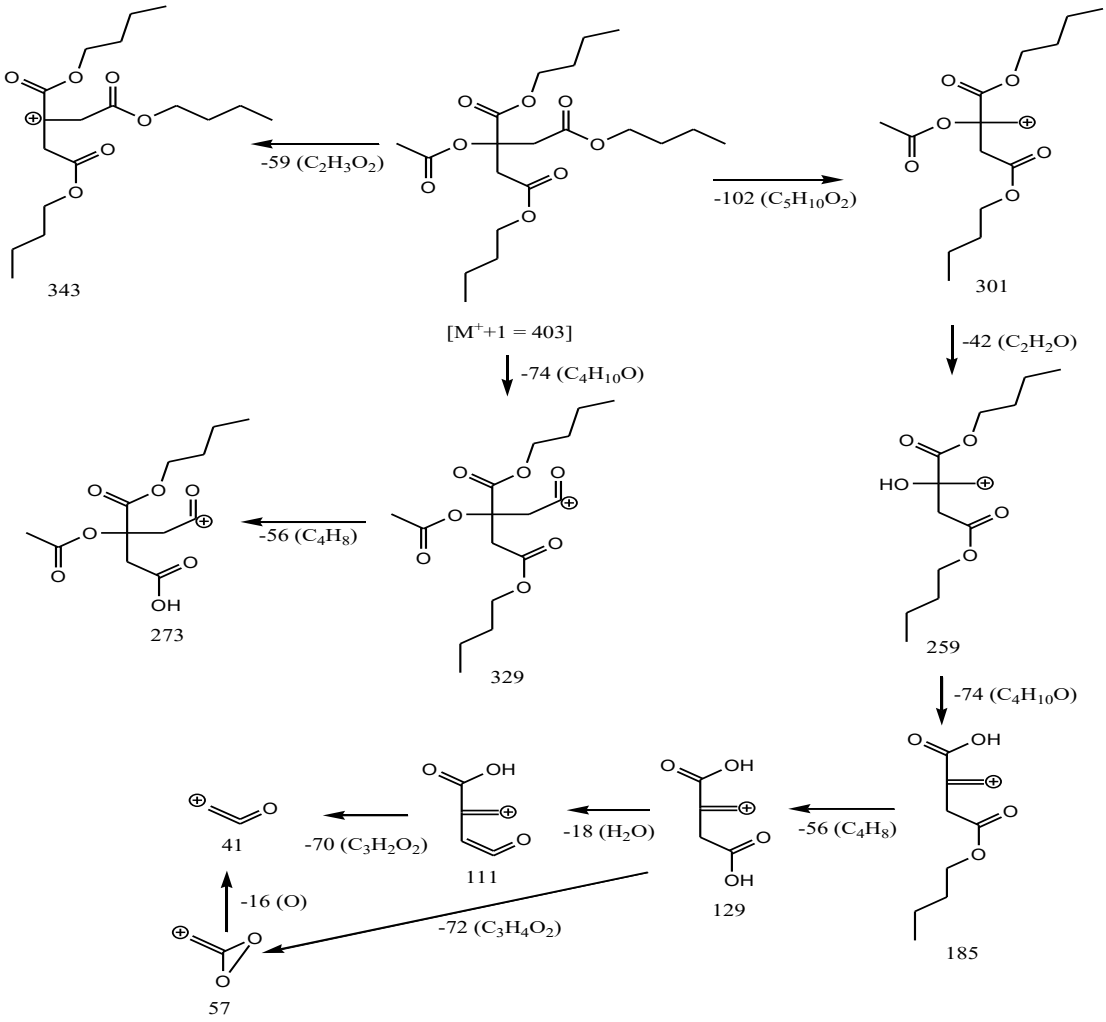

Fig. 7. *Fragmentation analysis of compound 1 in HE3 fraction of Michelia champaca bark extract*

Table 3. Fragmentation analysis of compound 1 in HE3 extract of *Michelia champaca* bark extract

Peak	Molecular	Fragment	Molecular Formula
403	(M^++1)	-	$C_{20}H_{34}O_8$
301	(M^++1)-102	$C_5H_{10}O_2$	$C_{15}H_{25}O_6$
259	(M^++1)-102-42	C_2H_2O	$C_{13}H_{23}O_5$
185	(M^++1)-102-42-74	$C_4H_{10}O$	$C_9H_{13}O_4$
129	(M^++1)-102-42-74-56	C_4H_8	$C_5H_5O_4$
111	(M^++1)-102-42-74-56-18	H_2O	$C_5H_3O_3$
41	(M^++1)-102-42-74-56-18-70	$C_3H_2O_2$	C_2HO

tract was composed of two compounds very difficult to separate using the Thin-layer chromatography (TLC) method. Compound 1 has a retention time of 14,290 with the molecular ion appearing as (M^++1) at m/z 403 (Fig. 5). Based on NIST08.LIB Library data, compound 1 was similar to the tributyl acetylcitrate compound, which has a molecular weight of 402 g/mole with a similarity percentage of 97% (Fig. 6). The molecular ion of compound 1 in the HE3 fraction appears as (M^++1) at m/z 403 with a base peak at m/z 185. The high percentage of similarity values strengthens the notion that compound 1 in the HE3 fraction was tributyl acetylcitrate. This is reinforced by the results of the fragmentation analysis of compound 1 as shown in Fig. 7 and Table 3. Based on the fragmentation results in Fig. 7 and Table 3, it is clear that compound 1 from the HE3 fraction is a tributyl acetylcitrate, which has the structural formula shown in Fig. 8. The fragmentation pattern of this compound is almost the same as the tributyl acetylcitrate found in *Adiantum capillus-veneris* where the molecular ion appears as (M^+) at m/z 402 with fragmentation peaks at m/z 329, 273, 259, 231, 213, 185, 157, 129, 112 and 57 with a basic peak at m/z 185 (Hussein *et al*., 2016) and in *Michelia alba* bark where the molecular ion appeared as (M^++1) at m/z 403 with fragmentation peaks at m/z 329, 273, 259, 231, 213, 185, 157,

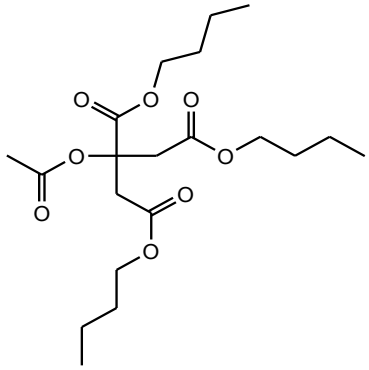

Fig. 8. *Structure of tributyl acetylcitrate*

Table 4. Fragmentation analysis of compound 2 in HE3 extract of *Michelia champaca* bark extract.

Peak	Molecular	Fragment	Molecular Formula
447	$(M^+ +1)$	-	$C_{28}H_{46}O_4$
279	$(M^+ +1)$-168	$C_{12}H_{24}$	$C_{16}H_{22}O_4$
261	$(M^+ +1)$-168-18	H_2O	$C_{16}H_{20}O_3$
149	$(M^+ +1)$-168-18-112	C_8H_{16}	$C_8H_4O_3$
104	$(M^+ +1)$-168-18-112-45	HCO_2	C_7H_3O
41	$(M^+ +1)$-168-18-112-45-63	C_5H_3	C_2O

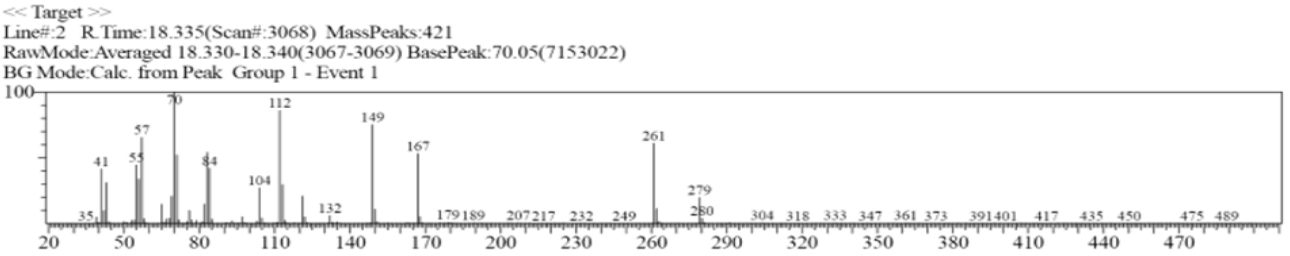

Fig. 9. *GC-MS spectrum of compound 2 in HE3 fraction of Michelia champaca bark extract*

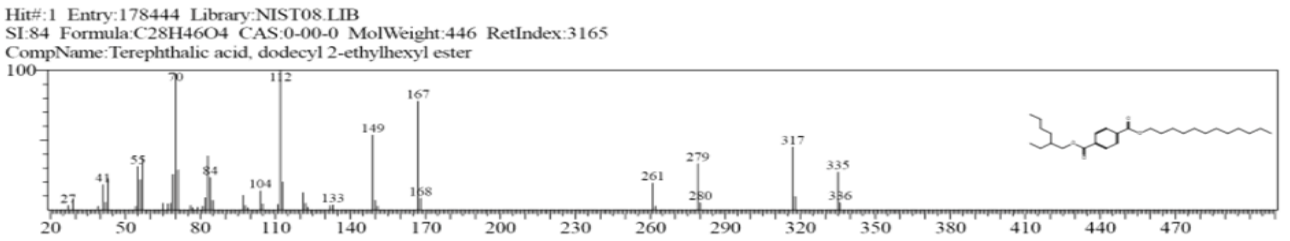

Fig. 10. *GC-MS spectrum of reference compound 2 in HE3 fraction of Michelia champaca bark extract*

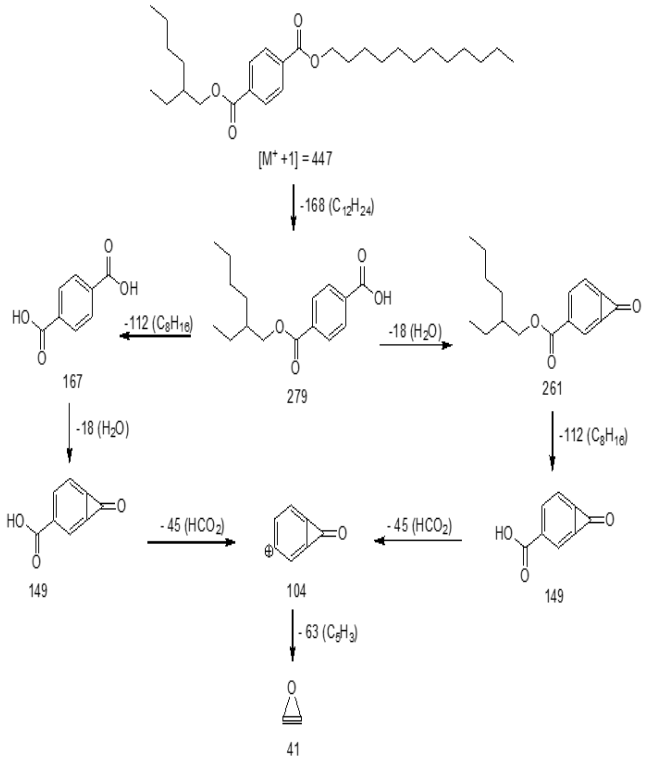

Fig. 11. *Fragmentation analysis of compound 2 in HE3 fraction of Michelia champaca bark extract*

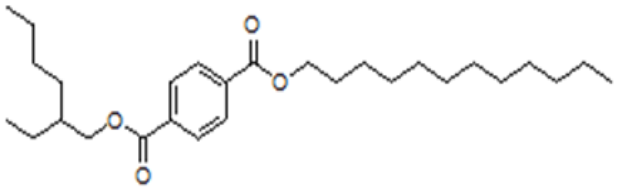

Fig. 12. *Structure of terephthalic acid, dodecyl-2-ethylhexyl ester*

129, 111, 57, and 43 with base peaks at m/z 185 (Bawa, 2019). This compound is strongly suspected of having a role in inhibiting the growth of the fungus *C. verruculosa* and is based on the results of research by Hussein *et al*. (2016), who found that the tributyl acetylcitrate compound is a component of a compound contained in the plant *Adiantum capillus-veneris* which exhibits antifungal activity against *Candida albicans* and *Fusarium* sp. at an extract concentration of 100 mg/ml.

Compound 2 in the HE3 fraction had a retention time of 18.334 min., with the GC-MS chromatogram shown in Fig. 9. Based on data from the NIST08.LIB Library. Compound 2 was similar to the terephthalic acid compound, dodecyl-2-ethylhexyl ester, which had a molecular weight of 446 g/mole with a similarity percentage of 84% (Fig. 10). The molecular ion of compound 2 in the

HE3 fraction had a value of m/z 447 with a base peak at m/z 70, did not appear. Because the percentage similarity between compound 2 and the reference compound was only 84%, a fragmentation analysis was carried out, which is shown in Fig. 11 and Table 4. Based on the fragmentation results in Fig. 11 and Table 4, it is clear that compound 2 from the HE3 fraction is a terephthalic acid, dodecyl-2-ethylhexyl ester, which has the structural formula as shown in Fig. 12. This compound is suspected of having a role in inhibiting the growth of the fungus *C. verruculosa* and is based on the results of research by Osuntokun and Cristina (2019) who found that the terephthalic acid, dodecyl-2-ethylhexyl ester contained in the essential oils from stem bark extract of *Spondias mombin* (Linn) which exhibited antifungal activity against *Aspergillus flavus* in extract concentration 10 mg/ml and antibacterial activity against *Bacillus subtilis* in extract concentration 5 mg/ml.

Conclusion

The methanol extract of *M. champaca* bark showed a very strong inhibitory effect on the growth of *C. verruculosa* fungi. The hexane extract of *M. champaca* bark also showed strong activity for *C. verruculosa* fungi. The hexane extract of the bark, especialy HE3 extract, contained two compounds: tributyl acetylcitrate and terephthalic acid, dodecyl-2-ethylhexyl ester, which can synergistically inhibit the growth of the fungus *C. verruculossa* very strongly. The results indicated that the bark extract of *M. champaca* had the potential to be a botanical fungicide.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Dean of Mathematics and Natural Sciences Faculty for funding this research.

Funding Source

This research was funded by the Faculty of Mathematics and Natural Sciences Udayana University Bali (Grant number: B/78.221/UN14.4.A/PT.01.03/2022)

Conflict of interests

The authors declare that they have no conflict of interest.

REFERENCES

1. Bawa I G. A. G. (2019). Active Compounds and Antifungal Activity of Bark Extract of *Michelia alba* to *Curvularia verruculosa* fungal caused Leaf Spot Disease in Rice (*Oryza sativa* L.) (Disertacion). Denpasar: Udayana University.
2. Bawa I G. A. G. (2021). Exploration of Plant Extracts have Antifungal Activity Against *Curvularia verruculosa* Fungi Causing Leaf Spot Disease in Rice Plants (*Oryza sativa* L.). *Journal of Chemistry*, 15(1), 8-15. DOI: https://doi.org/10.24843/JCHEM.2021v15.i01.p02.
3. Bawa I G. A. G. & Bogoriani N. W. (2021). Antifungal Activity of the Bark Extract of *Michelia Alba* against *Curvularia Verruculosa* Fungal the Cause of Leaf Spot Disease on Rice. *Biomedical & Pharmacology Journal*, 14(3), 1655-1659.DOI: https://dx.doi.org/10.13005/bpj/2266
4. Huang C. T, Chen S. J, Wu H. M, Kang Y. F, Chen H. L, Li W. J, Li H. T. & Chen C. Y. (2014). Chemical Constituents of the Stems of *Michelia champaca*. *Chemistry of Natural Compounds*, 50(6), 1047-1049. DOI: 10.1007/s10600-014-1156-9
5. Hussein H. M, Hameed I. H. & Ibraheem O. A. (2016). Antimicrobial Activity and Spectral Chemical Analysis of Methanolic Leaves Extract of *Adiantum Capillus-Veneris* Using GC-MS and FTIR Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*, 8 (3), 369-385.
6. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R. & Samiyappan R. (2005). Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas* pv oryzae. *Physiological and Mole. Plant Pathol.,* 65, 91-100. DOI: https://doi.org/10.1016/j.pmpp.2004.11.008
7. Kumar V. R, Kumar S, Shashidhara S, Anitha S. & Manjula M. (2011). Antioxidant and Antimicrobial Activities of Various Extracts of *Michelia champaca* Linn Flowers. *Deccan J. Pharmacology*, 2(1), 28-39.
8. Mangang H. C. & Chhetry G. K. N. (2012). Antifungal Properties of Certain Plant Extracts Against *Rhizoctonia solani* Causing Root Rot of French Bean in Organic Soil of Manipur. *International Journal of Scientific and Reasearch Publications*, 2(5), 1-4.
9. Omezzine F, Daami-Remadi M, Rinez A, Ladhari A. & Haouala R. (2011). In vitro assessment of *Inula* spp. organic extracts for their antifungal activity against some pathogenic and antagonistic fungi. *African Journal of Microbiology Research*, 5(21), 3527-3531.
10. Osuntokun O. T. & Cristina G. M. (2019). Bio Isolation, Chemical Purification, Identification, Antimicrobial and Synergistic Efficacy of Extracted Essential Oils from Stem Bark Extract of *Spodias mombin* (Linn). *International Journal of Molecular Biology*, 4(4), 135-143. DOI: 10.15406/ijmboa.2019.04.00110
11. Pawar B. T. (2015). Antifungal Activity of Some Latex Against Seed-Borne Pathogenic Fungi. *J. Biol. Chem. Research*, 32(1), 183-187.
12. West J. S, Bravo C, Oberit R, Lemaire D, Moshou D. & McCartney H. A. (2003). The Potential of Optical Canopy Measurement for Targeted Control of Field Crop Diseases. *Annual Review of Phytopathology*, 41, 593–614. DOI: 10.1146/annurev.phyto.41.121702.103726
13. Yoon M. Y, Cha B, & Kim Jin-Cheol. (2013). Recent Trends in Studies on Botanical Fungicides in Agriculture, *Plant Pathol J*., 29, 1–9. DOI: 10.5423/PPJ.RW.05.2012.0072